



US010967042B2

(12) **United States Patent**
Guerlavais et al.(10) **Patent No.: US 10,967,042 B2**(45) **Date of Patent: *Apr. 6, 2021**(54) **PEPTIDOMIMETIC MACROCYCLES**USPC 514/19.9
See application file for complete search history.(71) Applicant: **Aileron Therapeutics, Inc.**, Watertown,
MA (US)(56) **References Cited**(72) Inventors: **Vincent Guerlavais**, Arlington, MA
(US); **Carl Elkin**, Arlington, MA (US);
Huw M. Nash, Lexington, MA (US);
Tomi K. Sawyer, Southborough, MA
(US); **Bradford J. Graves**, Nutley, NJ
(US); **Eric Feyfant**, Lexington, MA
(US)

U.S. PATENT DOCUMENTS

6,686,148	B1	2/2004	Shen et al.
7,115,372	B2	10/2006	Shen et al.
7,192,713	B1	3/2007	Verdine et al.
7,202,332	B2	4/2007	Arora et al.
7,425,542	B2	9/2008	Maggio
7,723,469	B2	5/2010	Walensky et al.
7,786,072	B2	8/2010	Verdine et al.
7,960,506	B2	6/2011	Nash
7,981,998	B2	7/2011	Nash
7,981,999	B2	7/2011	Nash
RE42,624	E	8/2011	Fraser
7,998,927	B2	8/2011	Maggio
8,071,541	B2	12/2011	Arora et al.
8,076,290	B2	12/2011	Maggio
8,084,022	B2	12/2011	Maggio
8,133,863	B2	3/2012	Maggio
8,173,594	B2	5/2012	Maggio
8,198,405	B2	6/2012	Walensky et al.
8,226,949	B2	7/2012	Maggio
8,324,428	B2	12/2012	Verdine et al.
8,592,377	B2	11/2013	Verdine et al.
8,889,632	B2	11/2014	Bernal et al.

(Continued)

(73) Assignee: **AILERON THERAPEUTICS, INC.**,
Watertown, MA (US)(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days.This patent is subject to a terminal dis-
claimer.(21) Appl. No.: **16/223,473**(22) Filed: **Dec. 18, 2018**(65) **Prior Publication Data**

US 2019/0343914 A1 Nov. 14, 2019

Related U.S. Application Data(63) Continuation of application No. 15/229,517, filed on
Aug. 5, 2016, now Pat. No. 10,213,477, which is a
continuation of application No. 14/498,063, filed on
Sep. 26, 2014, now Pat. No. 9,505,804, which is a
continuation of application No. 13/767,852, filed on
Feb. 14, 2013, now Pat. No. 8,927,500.(60) Provisional application No. 61/723,770, filed on Nov.
7, 2012, provisional application No. 61/656,962, filed
on Jun. 7, 2012, provisional application No.
61/599,328, filed on Feb. 15, 2012.(51) **Int. Cl.**

A61K 38/10	(2006.01)
A61K 38/12	(2006.01)
C07K 7/08	(2006.01)
A61K 38/03	(2006.01)
C07K 7/06	(2006.01)
A61K 38/08	(2019.01)
C07K 1/113	(2006.01)
C07K 14/47	(2006.01)
C07K 7/54	(2006.01)
A61K 45/06	(2006.01)

(52) **U.S. Cl.**CPC **A61K 38/10** (2013.01); **A61K 38/03**
(2013.01); **A61K 38/08** (2013.01); **A61K 45/06**
(2013.01); **C07K 1/113** (2013.01); **C07K 7/06**
(2013.01); **C07K 7/08** (2013.01); **C07K 7/54**
(2013.01); **C07K 14/4746** (2013.01); **A61K**
38/12 (2013.01)(58) **Field of Classification Search**CPC **A61K 38/12**; **A61K 38/00**; **A61K 38/10**;
C07K 7/56; **C07K 14/001**

FOREIGN PATENT DOCUMENTS

AU	2008232709	A1	10/2008
CA	2700925	A1	4/2009

(Continued)

OTHER PUBLICATIONS

Kallen, et al., "Crystal Structures of Human MdmX (HdmX) in
Complex with p53 Peptide Analogues Reveal Surprising Conformational
Changes", The Journal of Biological Chemistry vol. 284, No. 13,
pp. 8812-8821, Mar. 27, 2009.

(Continued)

Primary Examiner — James H Alstrum-Acevedo*Assistant Examiner* — Kaipeen E Yang(74) *Attorney, Agent, or Firm* — Wilson Sonsini Goodrich
& Rosati(57) **ABSTRACT**

Provided herein are peptidomimetic macrocycles containing amino acid sequences with at least two modified amino acids that form an intramolecular cross-link that can help to stabilize a secondary structure of the amino acid sequence. Suitable sequences for stabilization include those with homology to the p53 protein. These sequences can bind to the MDM2 and/or MDMX proteins. Also provided herein are methods of using such macrocycles for the treatment of diseases and disorders, such as cancers or other disorders characterized by a low level or low activity of a p53 protein or high level of activity of a MDM2 and/or MDMX protein.

18 Claims, 7 Drawing Sheets**Specification includes a Sequence Listing.**

(56)

References Cited

U.S. PATENT DOCUMENTS

2005/0250680 A1 11/2005 Walensky et al.
 2009/0149630 A1 6/2009 Walensky et al.
 2009/0176964 A1 7/2009 Walensky et al.
 2009/0275519 A1 11/2009 Nash et al.
 2009/0326192 A1 12/2009 Nash et al.
 2010/0081611 A1 4/2010 Bradner et al.
 2010/0273704 A1 10/2010 Korsmeyer et al.
 2011/0144303 A1 6/2011 Nash et al.
 2011/0144306 A1 6/2011 Verdine et al.
 2011/0218155 A1 9/2011 Walensky et al.
 2011/0223149 A1 9/2011 Nash et al.
 2011/0245175 A1 10/2011 Arora et al.
 2011/0250685 A1 10/2011 Nash
 2012/0082636 A1 4/2012 Walensky et al.
 2012/0101047 A1 4/2012 Nash et al.
 2012/0115783 A1 5/2012 Nash et al.
 2012/0115793 A1 5/2012 Nash et al.
 2012/0178700 A1 7/2012 Nash et al.
 2012/0270800 A1 10/2012 Verdine et al.
 2013/0005943 A1 1/2013 Arora et al.

FOREIGN PATENT DOCUMENTS

CN 1906209 A 1/2007
 CN 101636407 A 1/2010
 CN 102223891 A 10/2011
 EP 2091552 A2 8/2009
 EP 2310407 A2 4/2011
 EP 2377849 A2 10/2011
 EP 2489360 A1 8/2012
 EP 2114428 B1 10/2012
 EP 2637680 A2 9/2013
 JP 2008501623 A 1/2008
 JP 2010519318 A 6/2010
 WO WO-2005044839 A2 5/2005
 WO WO-2005044839 A3 7/2005
 WO WO-2005118620 A2 12/2005
 WO WO-2008061192 A2 5/2008
 WO WO-2008076904 A1 6/2008
 WO WO-2008061192 A3 7/2008
 WO WO-2008095063 A1 8/2008
 WO WO-2008104000 A2 8/2008
 WO WO-2008121767 A2 10/2008
 WO WO-2008137633 A2 11/2008
 WO WO-2008121767 A3 1/2009
 WO WO-2009042237 A2 4/2009
 WO WO-2009099677 A2 8/2009
 WO WO-2009110952 A2 9/2009
 WO WO-2009126292 A2 10/2009
 WO WO-2009042237 A3 12/2009
 WO WO-2009149214 A2 12/2009
 WO WO-2010011313 A2 1/2010
 WO WO-2010033879 A2 3/2010
 WO WO-2010034026 A1 3/2010
 WO WO-2010034028 A1 3/2010
 WO WO-2010034029 A1 3/2010
 WO WO-2010034031 A1 3/2010
 WO WO-2010034032 A2 3/2010
 WO WO-2010034034 A1 3/2010
 WO WO-2010060112 A1 5/2010
 WO WO-2010068684 A2 6/2010
 WO WO-2010083347 A2 7/2010
 WO WO-2010011313 A3 12/2010
 WO WO-2011008260 A2 1/2011
 WO WO-2011008260 A3 3/2011
 WO WO-2011038049 A1 3/2011
 WO WO-2011047215 A1 4/2011
 WO WO-2012016186 A1 2/2012
 WO WO-2012021874 A1 2/2012
 WO WO-2012021875 A1 2/2012
 WO WO-2012021876 A2 2/2012
 WO WO-2012040459 A2 3/2012
 WO WO-2012065181 A2 5/2012
 WO WO-2012040459 A3 6/2012

WO WO-2012122059 A1 9/2012
 WO WO-2012173846 A2 12/2012
 WO WO-2012174423 A1 12/2012

OTHER PUBLICATIONS

Al-Lazikani, et al. Combinatorial drug therapy for cancer in the post-genomic era. *Nature biotechnology* 30.7 (2012): 679-692.
 Andrews et al. Forming Stable Helical Peptide Using Natural and Artificial Amino Acids. *Tetrahedron*. 1999;55:11711-11743.
 Angell, et al. Peptidomimetics via copper-catalyzed azide-alkyne cycloadditions. *Chem Soc Rev*. Oct. 2007;36(10):1674-89.
 Arosio, et al. Click chemistry to functionalise peptidomimetics. *Tetrahedron Letters*. 2006; 47:3697-3700.
 Bernal et al., Reactivation of the p53 tumor suppressor pathway by a stapled p53 peptide. (2007) *J. Am Chem Soc*. 9129, 2456-2457.
 Bernal, et al. (2010). A stapled p53 helix overcomes HDMX-mediated suppression of p53. *Cancer Cell* 18, 411-422.
 Bernal, et al. A stapled p53 helix overcomes HDMX-mediated suppression of p53. *Cancer Cell*. Nov. 16, 2010;18(5):411-22. doi: 10.1016/j.ccr.2010.10.024.
 Bernal, et al. Reactivation of the p53 tumor suppressor pathway by a stapled p53 peptide. *J Am Chem Soc*. Mar. 7, 2007;129(9):2456-7.
 Blackwell, et al. Highly Efficient Synthesis of Covalently Cross-Linked Peptide Helices by Ring-Closing Metathesis. *Angewandte Chemie International Edition*. 1998; 37(23):3281-3284.
 Blackwell, et al. Ring-closing metathesis of olefinic peptides: design, synthesis, and structural characterization of macrocyclic helical peptides. *J Org Chem*. Aug. 10, 2001;66(16):5291-302.
 Brown, et al. A spirologomer α -helix mimic that binds HDM2, penetrates human cells and stabilizes HDM2 in cell culture. *PLoS One*. 2012;7(10):e45948. doi: 10.1371/journal.pone.0045948. Epub Oct. 18, 2012.
 Brown, et al. Stapled peptides with improved potency and specificity that activate p53. *ACS Chem Biol*. Mar. 15, 2013;8(3):506-12. doi: 10.1021/cb3005148. Epub Dec. 18, 2012.
 Cantel, et al. Synthesis and Conformational Analysis of a Cyclic Peptide Obtained via i to i+4 Intramolecular Side-Chain to Side-Chain Azide-Alkyne 1,3-Dipolar Cycloaddition. *JOC Featured Article*. Published on the web May 20, 2008.
 Chène, P., "Inhibiting the p53-MDM2 Interaction: An Important Target for Cancer Therapy," *Nat Rev. Cancer* 3:102-109 (2003).
 Clavier, et al. Ring-closing metathesis in biphasic BMI.PF6 ionic liquid/toluene medium: a powerful recyclable and environmentally friendly process. *Chem Commun (Camb)*. Oct. 21, 2004;(20):2282-3. Epub Aug. 25, 2004.
 Conrad, et al. Ruthenium-Catalyzed Ring-Closing Metathesis: Recent Advances, Limitations and Opportunities. *Current Organic Chemistry*. Jan. 2006; vol. 10, No. 2, 10(2):185-202(18).
 Co-pending U.S. Appl. No. 13/494,846, filed Jun. 12, 2012.
 Co-pending U.S. Appl. No. 13/655,442, filed Oct. 18, 2010.
 File Hcaplus on STN. AN Number: 1979:168009. Greenlee et al. A general synthesis of alpha-vinyl-alpha-amino acids *Tetrahedron Letters* (1978), (42), 3999-4002. Abstract date 1984.
 Fischer, P. Peptide, Peptidomimetic, and Small-molecule Antagonists of the p53-HDM2 Protein-Protein Interaction. *Int J Pept Res Ther*. Mar. 2006;12(1):3-19. Epub Mar. 15, 2006.
 Grubbs, et al. Ring-Closing Metathesis and Related Processes in Organic Synthesis. *Acc. Chem. Res.*, 1995, 28 (11), pp. 446-452.
 Guo et al., Probing the alpha-helical structural stability of stapled p53 peptides: molecular dynamics simulations and analysis. *Chem Biol Drug Des*. Apr. 2010;75(4):348-59. doi: 10.1111/j.1747-0285.2010.00951.x.
 Hong, et al. Efficient removal of ruthenium byproducts from olefin metathesis products by simple aqueous extraction. *Org Lett*. May 10, 2007;9(10):1955-7.
 Invitation to Pay Additional Fees for PCT/US2011/052755 dated Feb. 16, 2012.
 Invitation to Pay Additional Fees for PCT/US2013/062004, dated Jan. 2, 2014.
 Isidro-Llobet, et al. Amino acid-protecting groups. *Chem Rev*. Jun. 2009;109(6):2455-504. doi: 10.1021/cr800323s.

(56)

References Cited

OTHER PUBLICATIONS

Knackmuss et al., Specific inhibition of interleukin-13 activity by a recombinant human single-chain immunoglobulin domain directed against the IL-13 receptor alpha chain. *Biol Chem. Mar.* 2007;388(3):325-30.

Kolb et al., Click Chemistry: Diverse Chemical Function from a Few Good Reactions. *Angew Chem Int Ed Engl.* Jun. 1, 2001;40(11):2004-2021.

Kotha et al., Modification of constrained peptides by ring-closing metathesis reaction. *Bioorg Med Chem Lett.* Jun. 4, 2001;11(11):1421-3.

Moses, et al. The growing applications of click chemistry. *Chem Soc Rev.* Aug. 2007;36(8):1249-62.

Office action dated Apr. 9, 2014 for U.S. Appl. No. 13/767,852.

Office action dated Nov. 5, 2002 for U.S. Appl. No. 09/574,086.

Office action dated Dec. 31, 2013 for U.S. Appl. No. 12/525,123.

Office Communication, dated Jan. 3, 2013, for U.S. Appl. No. 12/593,384.

Schafmeister et al. An all-hydrocarbon crosslinking system for enhancing the helicity and metabolic stability of peptides. *J. Am Chem. Soc.* 2000;122:5891-5892.

Shangary, et al. Targeting the MDM2-p53 interaction for cancer therapy. *Clin Cancer Res.* Sep. 1, 2008;14(17):5318-24. doi: 10.1158/1078-0432.CCR-07-5136.

Verdine et al. The challenge of drugging undruggable targets in cancer: lessons learned from targeting BCL-2 family members. *Clin Cancer Res.* 13(24):7264-7270 (2007).

Verdine et al., Stapled peptides for intracellular drug targets. *Methods Enzymol.* 2012;503:3-33. doi: 10.1016/B978-0-12-396962-0.00001-X.

Walensky et al., A stapled BID BH3 helix directly binds and activates BAX. (2006) *Mol Cell* 24:199-210.

Walensky, et al. A stapled BID BH3 helix directly binds and activates BAX. *Mol. Cell.* Oct. 20, 2006;24(2):199-210.

Walensky, et al. Activation of apoptosis in vivo by a hydrocarbon-stapled BH3 helix. *Science.* Sep. 3, 2004;305(5689):1466-70.

Walensky, et al. Activation of apoptosis in vivo by a hydrocarbon-stapled BH3 helix. *Science.* 2004;305(5689):1466-1470.

Figure 1

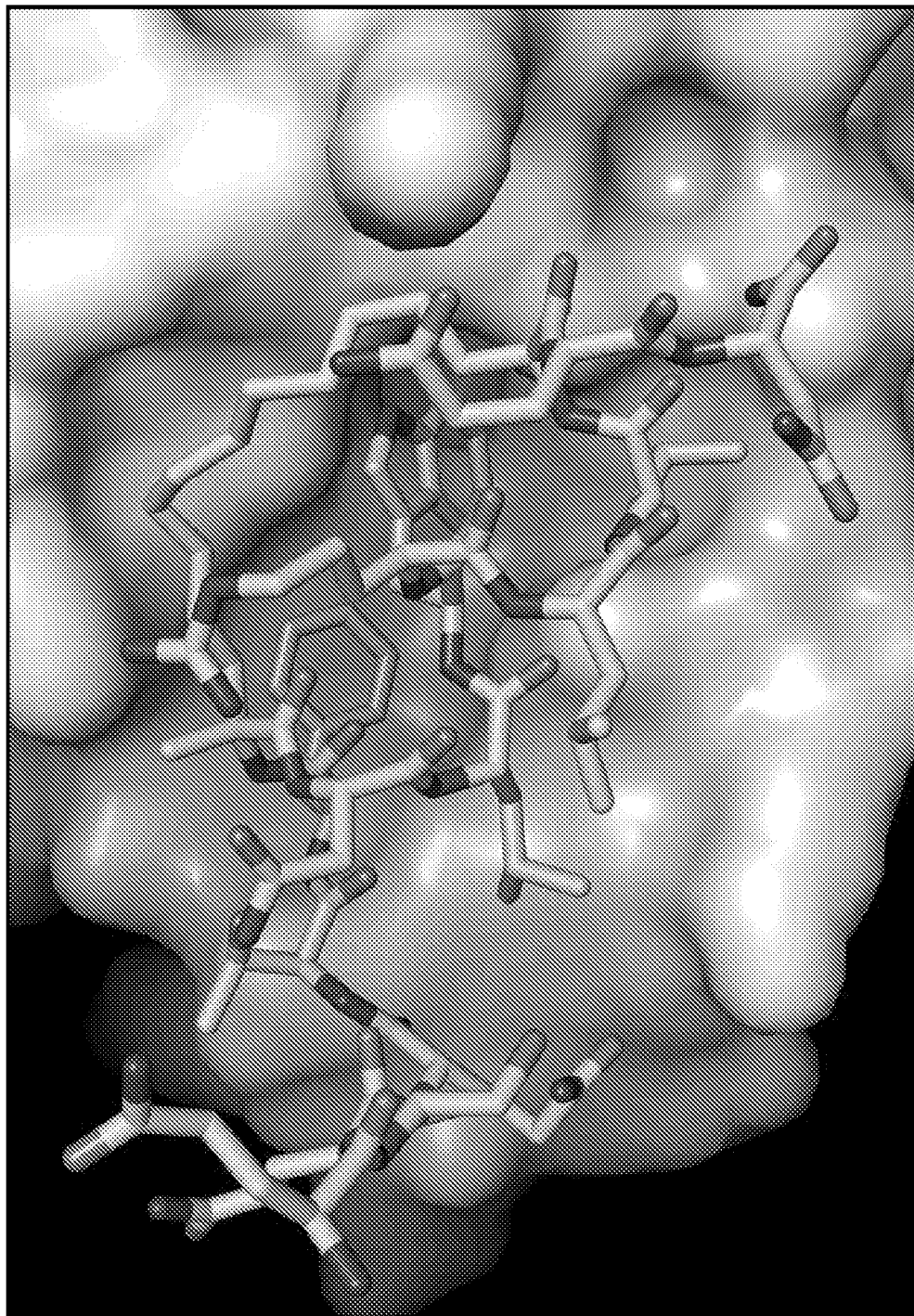


Figure 2

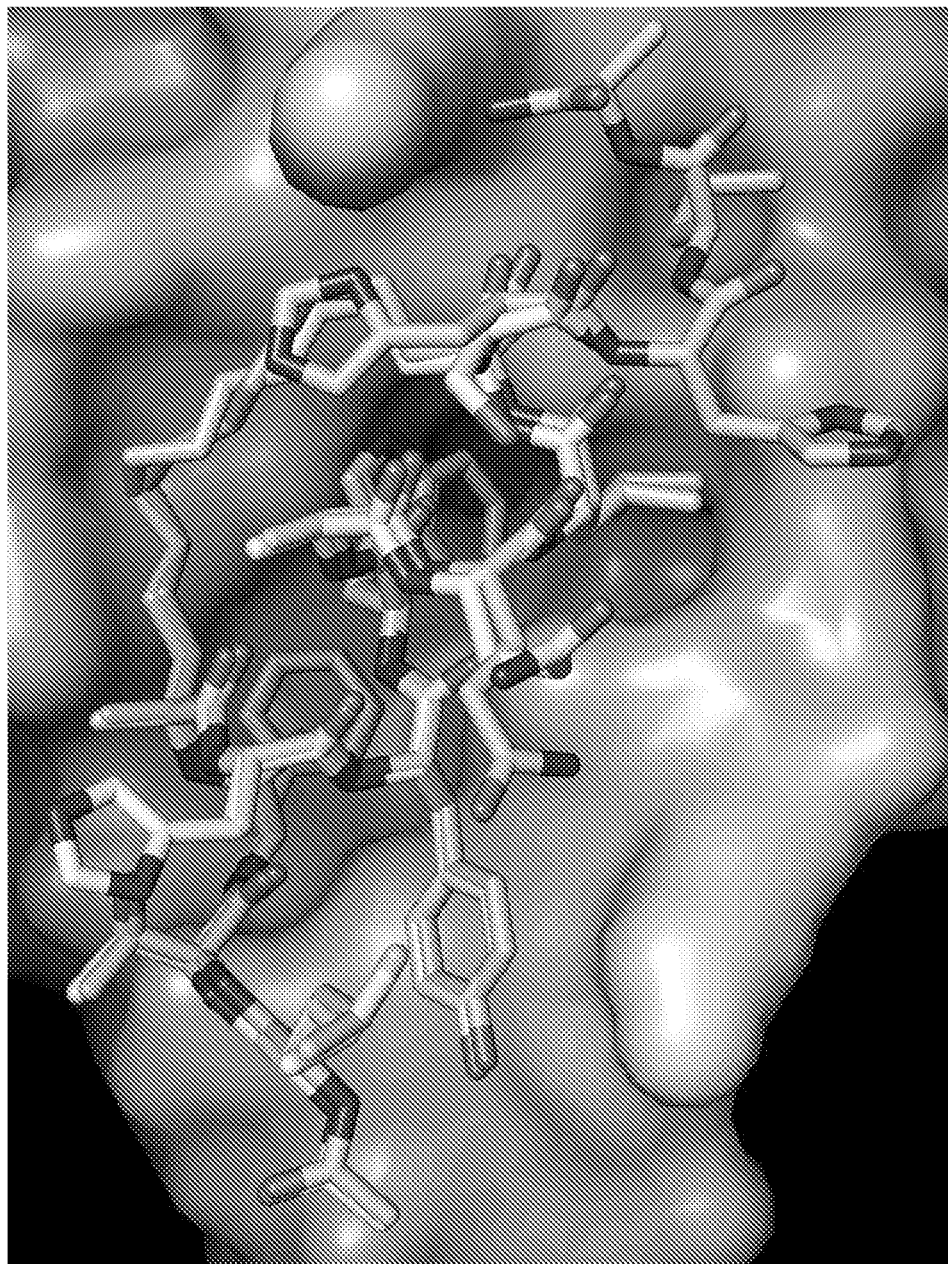


Figure 3

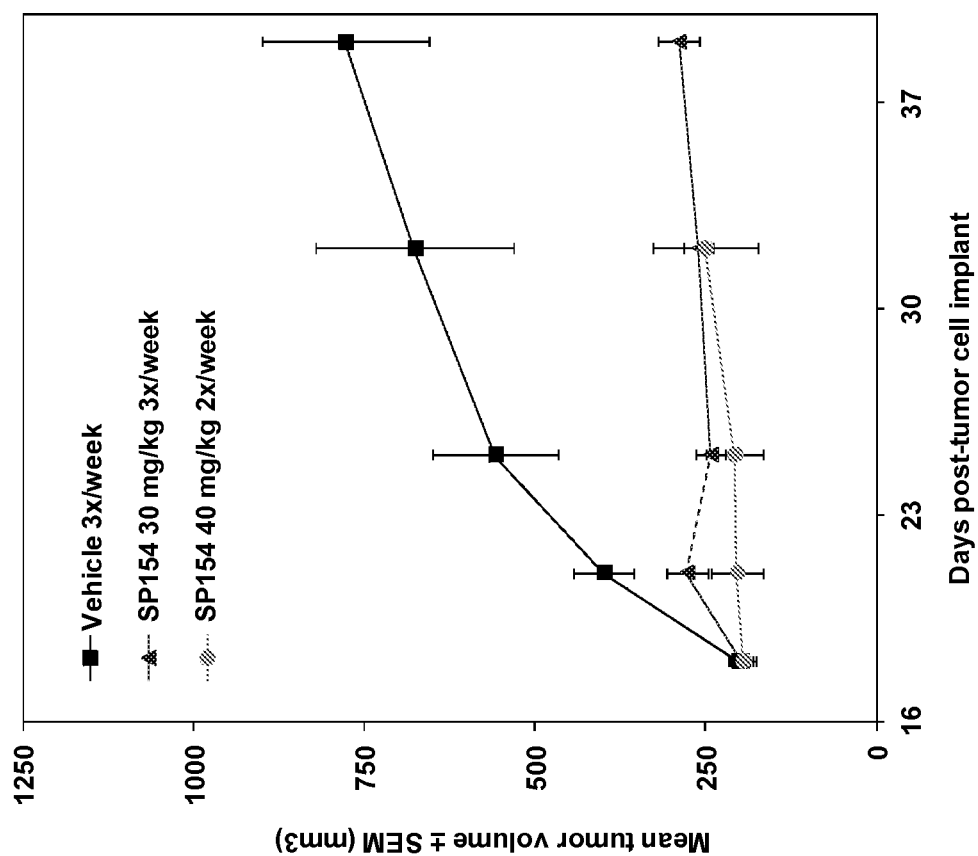


Figure 4

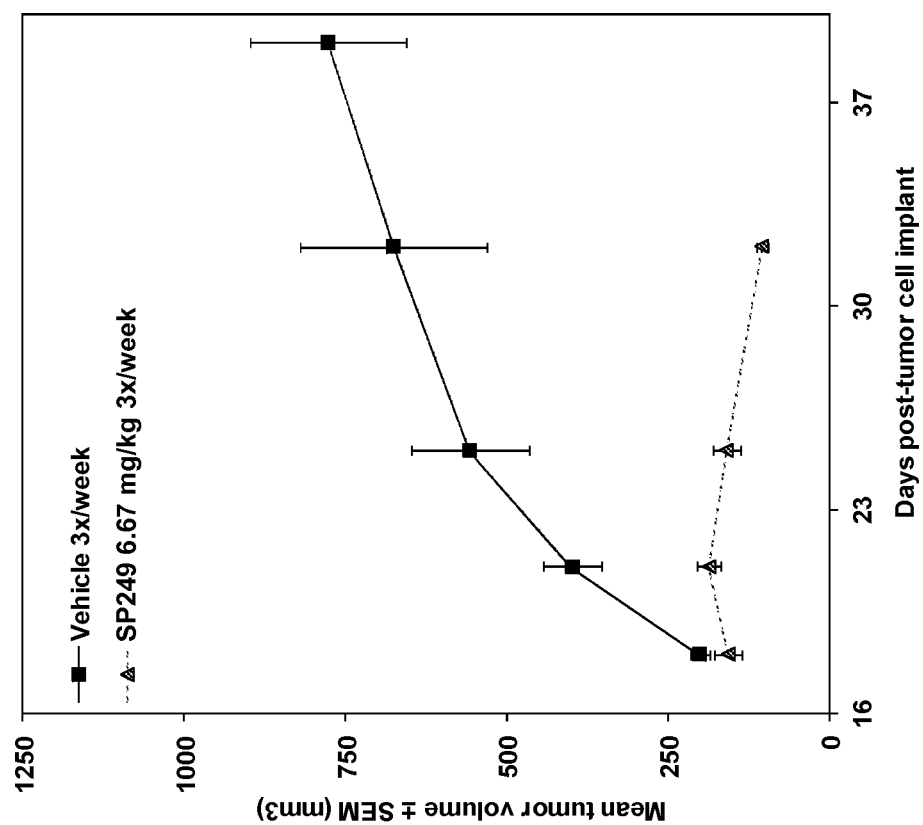


Figure 5

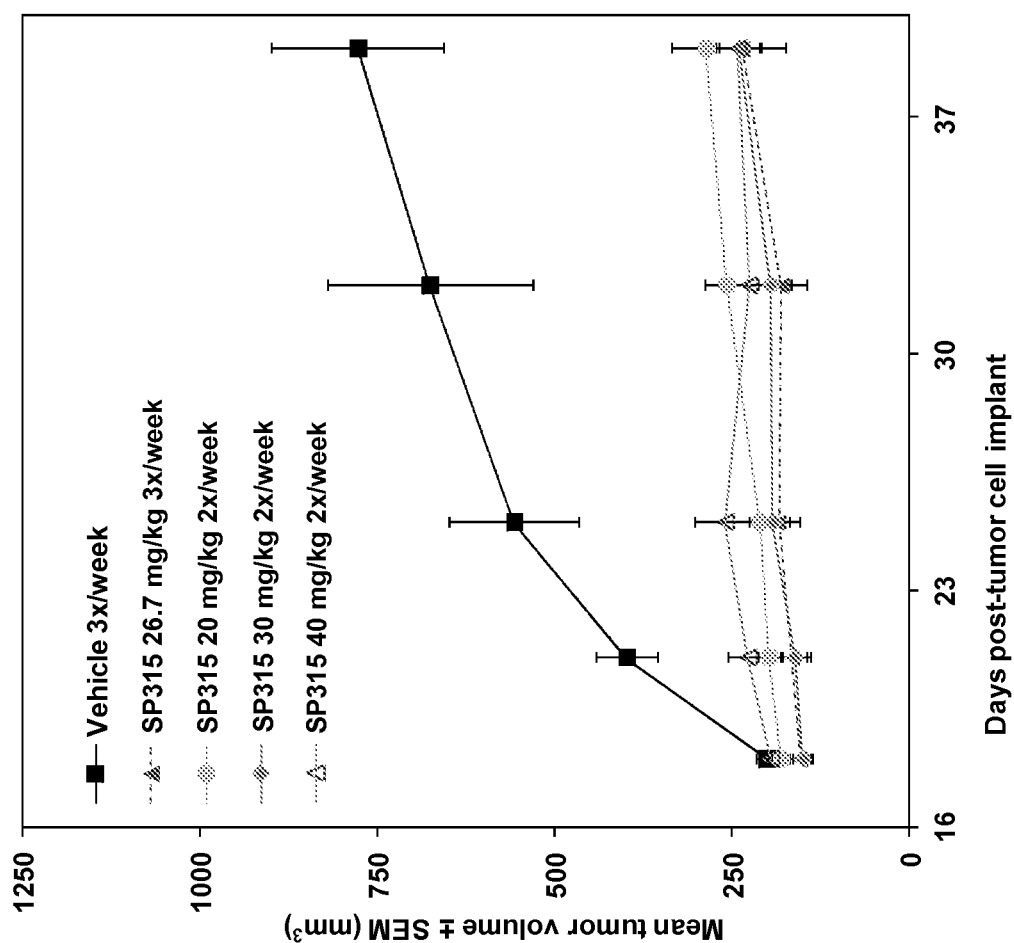


Figure 6

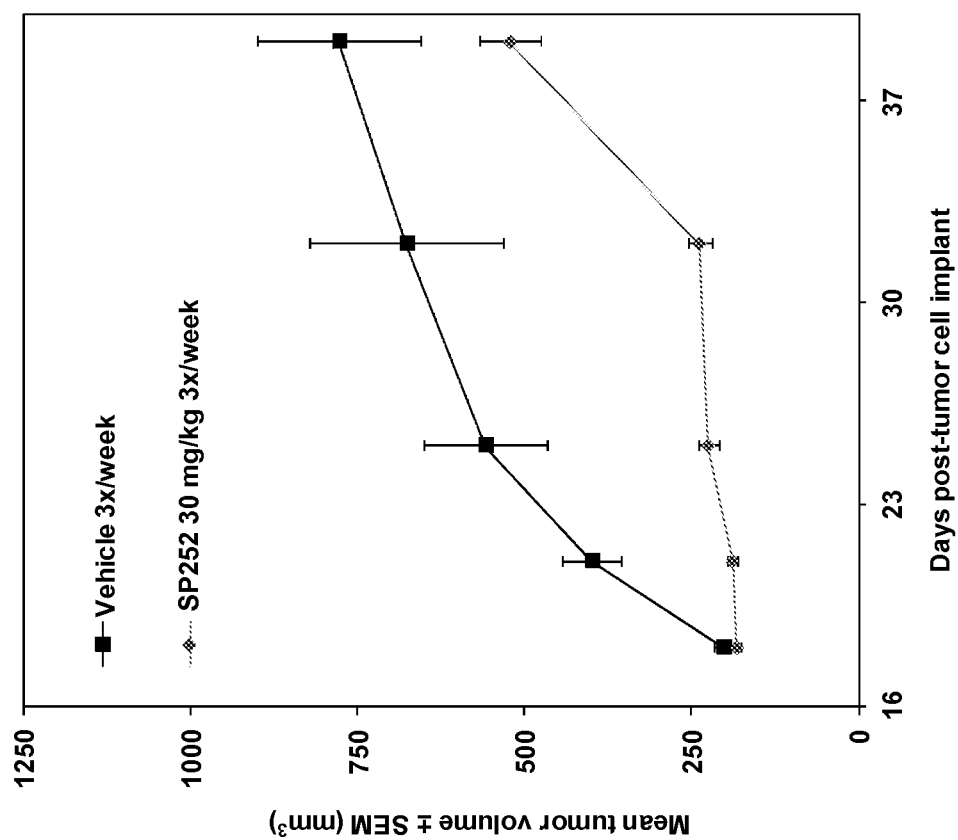
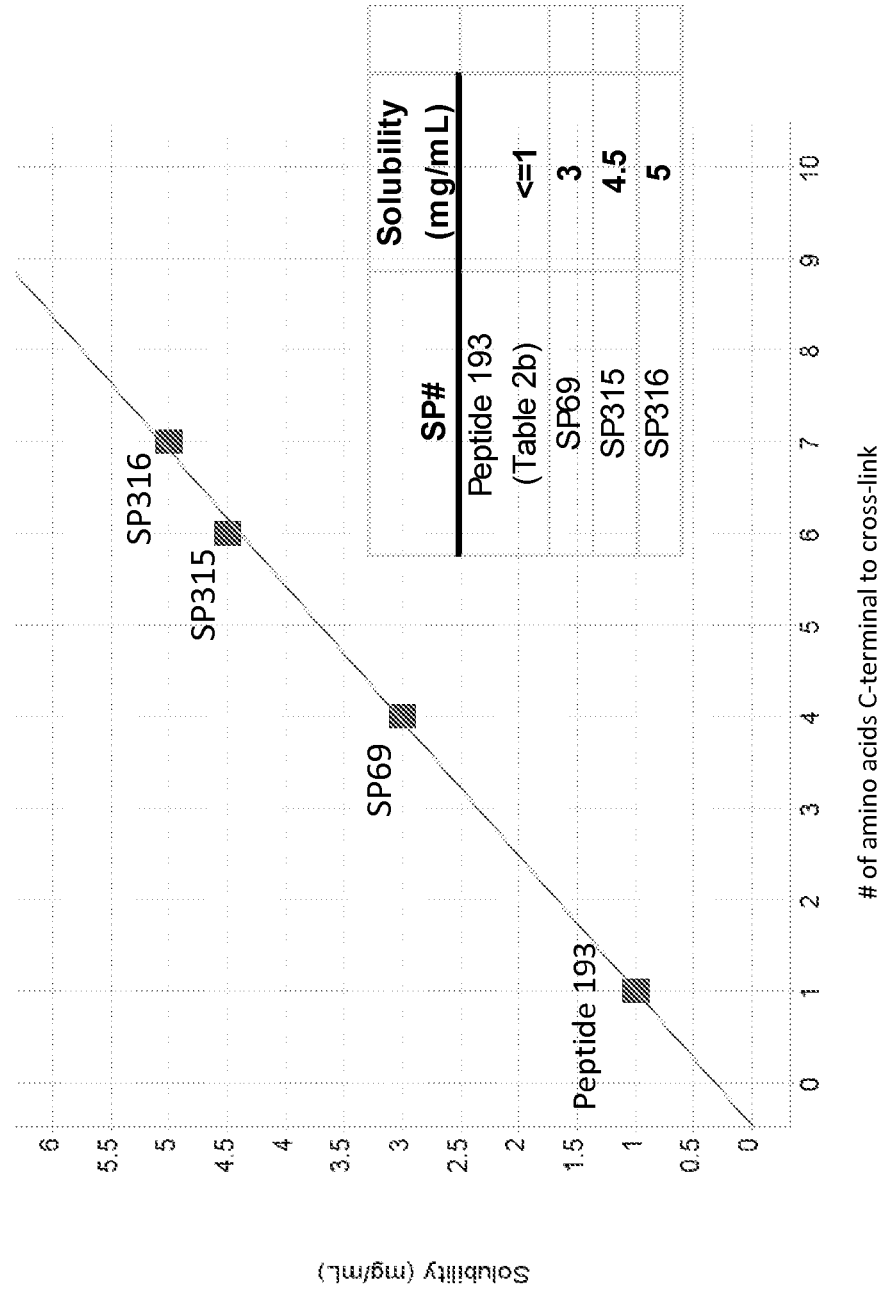


Figure 7



PEPTIDOMIMETIC MACROCYCLES

CROSS-REFERENCE

This application is a continuation application of continuation U.S. application Ser. No. 15/229,517, filed Aug. 5, 2016, now U.S. Pat. No. 10,213,477, issued Feb. 26, 2019, which is a continuation application of U.S. application Ser. No. 14/498,063, filed Sep. 26, 2014, now U.S. Pat. No. 9,505,804, issued Nov. 29, 2016, which is a continuation application of U.S. application Ser. No. 13/767,852, filed Feb. 14, 2013, now U.S. Pat. No. 8,927,500, issued Jan. 6, 2015, which claims the benefit of U.S. Provisional Application No. 61/723,770, filed Nov. 7, 2012, U.S. Provisional Application No. 61/656,962, filed Jun. 7, 2012, and U.S. Provisional Application No. 61/599,328, filed Feb. 15, 2012; each of which is incorporated herein by reference in their entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Dec. 12, 2018, is named 35224-766.303_SL.txt and is 1,888,256 bytes in size.

BACKGROUND OF THE INVENTION

The human transcription factor protein p53 induces cell cycle arrest and apoptosis in response to DNA damage and cellular stress, and thereby plays a critical role in protecting cells from malignant transformation. The E3 ubiquitin ligase MDM2 (also known as HDM2) negatively regulates p53 function through a direct binding interaction that neutralizes the p53 transactivation activity, leads to export from the nucleus of p53 protein, and targets p53 for degradation via the ubiquitylation-proteasomal pathway. Loss of p53 activity, either by deletion, mutation, or MDM2 overexpression, is the most common defect in human cancers. Tumors that express wild type p53 are vulnerable to pharmacologic agents that stabilize or increase the concentration of active p53. In this context, inhibition of the activities of MDM2 has emerged as a validated approach to restore p53 activity and resensitize cancer cells to apoptosis in vitro and in vivo. MDMX (MDM4) has more recently been identified as a similar negative regulator of p53, and studies have revealed significant structural homology between the p53 binding interfaces of MDM2 and MDMX. The p53-MDM2 and p53-MDMX protein-protein interactions are mediated by the same 15-residue alpha-helical transactivation domain of p53, which inserts into hydrophobic clefts on the surface of MDM2 and MDMX. Three residues within this domain of p53 (F19, W23, and L26) are essential for binding to MDM2 and MDMX.

There remains a considerable need for compounds capable of binding to and modulating the activity of p53, MDM2 and/or MDMX. Provided herein are p53-based peptidomimetic macrocycles that modulate an activity of p53. Also provided herein are p53-based peptidomimetic macrocycles that inhibit the interactions between p53, MDM2 and/or MDMX proteins. Further, provided herein

are p53-based peptidomimetic macrocycles that can be used for treating diseases including but not limited to cancer and other hyperproliferative diseases.

SUMMARY OF THE INVENTION

Described herein are stably cross-linked peptides related to a portion of human p53 ("p53 peptidomimetic macrocycles"). These cross-linked peptides contain at least two modified amino acids that together form an intramolecular cross-link that can help to stabilize the alpha-helical secondary structure of a portion of p53 that is thought to be important for binding of p53 to MDM2 and for binding of p53 to MDMX. Accordingly, a cross-linked polypeptide described herein can have improved biological activity relative to a corresponding polypeptide that is not cross-linked. The p53 peptidomimetic macrocycles are thought to interfere with binding of p53 to MDM2 and/or of p53 to MDMX, thereby liberating functional p53 and inhibiting its destruction. The p53 peptidomimetic macrocycles described herein can be used therapeutically, for example to treat cancers and other disorders characterized by an undesirably low level or a low activity of p53, and/or to treat cancers and other disorders characterized by an undesirably high level of activity of MDM2 or MDMX. The p53 peptidomimetic macrocycles can also be useful for treatment of any disorder associated with disrupted regulation of the p53 transcriptional pathway, leading to conditions of excess cell survival and proliferation such as cancer and autoimmunity, in addition to conditions of inappropriate cell cycle arrest and apoptosis such as neurodegeneration and immune deficiencies. In some embodiments, the p53 peptidomimetic macrocycles bind to MDM2 (e.g., GenBank® Accession No.: 228952; GI:228952) and/or MDMX (also referred to as MDM4; GenBank® Accession No.: 88702791; GI:88702791).

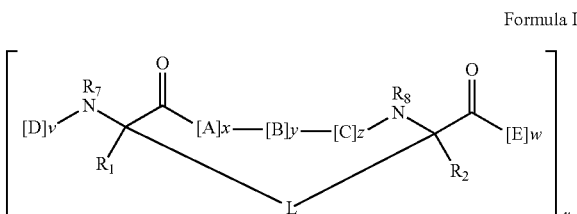
In one aspect, provided herein is a peptidomimetic macrocycle comprising an amino acid sequence which is at least about 60%, 80%, 90%, or 95% identical to an amino acid sequence chosen from the group consisting of the amino acid sequences in Table 1, Table 1a, Table 1b, or Table 1c. Alternatively, an amino acid sequence of said peptidomimetic macrocycle is chosen from the group consisting of the amino acid sequences in Table 4. In some embodiments, the peptidomimetic macrocycle is not a peptide as shown in Table 2a or 2b. In other cases, the peptidomimetic macrocycle does not comprise a structure as shown in Table 2a or 2b. In some embodiments, the peptidomimetic macrocycle has an amino acid sequence chosen from Table 1. In some embodiments, the peptidomimetic macrocycle has an amino acid sequence chosen from Table 1a. In some embodiments, the peptidomimetic macrocycle has an amino acid sequence chosen from Table 1b. In some embodiments, the peptidomimetic macrocycle has an amino acid sequence chosen from Table 1c.

Alternatively, an amino acid sequence of said peptidomimetic macrocycle is chosen as above, and further wherein the macrocycle does not include a thioether or a triazole. In some embodiments, the peptidomimetic macrocycle comprises a helix, such as an α -helix. In other embodiments, the peptidomimetic macrocycle comprises an α,α -disubstituted

3

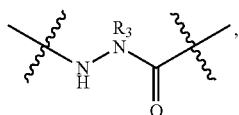
amino acid. A peptidomimetic macrocycle can comprise a crosslinker linking the α -positions of at least two amino acids. At least one of said two amino acids can be an α,α -disubstituted amino acid.

In some embodiments, provided are peptidomimetic macrocycle of the formula:



wherein:

each A, C, D, and E is independently an amino acid;
B is an amino acid,



[—NH—L₃—CO—], [—NH—L₃—SO₂—], or [—NH—L₃—];

R₁ and R₂ are independently —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo-; or at least one of R₁ and R₂ forms a macrocycle-forming linker L' connected to the alpha position of one of said D or E amino acids;

R₃ is hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R₅;

each L or L' is independently a macrocycle-forming linker of the formula —L₁—L₂—;

L₁ and L₂ and L₃ are independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, cycloarylene, heterocycloarylene, or [—R₄—K—R₄—]_n, each being optionally substituted with R₅;

each R₄ is alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene;

each K is O, S, SO, SO₂, CO, CO₂, or CONR₃;

each R₅ is independently halogen, alkyl, —OR₆, —N(R₆)₂, —SR₆, —SOR₆, —SO₂R₆, —CO₂R₆, a fluorescent moiety, a radioisotope or a therapeutic agent;

each R₆ is independently —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heterocycloalkyl, a fluorescent moiety, a radioisotope or a therapeutic agent;

R₇ is —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R₅, or part of a cyclic structure with a D residue;

R₈ is —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R₅, or part of a cyclic structure with an E residue;

v and w are independently integers from 1-1000, for example 1-500, 1-200, 1-100, 1-50, 1-30, 1-20, or 1-10;

u is an integer from 1-10, for example 1-5, 1-3 or 1-2;

4

x, y and z are independently integers from 0-10, for example the sum of x+y+z is 2, 3, or 6; and

n is an integer from 1-5.

In some embodiments, w>2 and each of the first two amino acid represented by E comprises an uncharged side chain or a negatively charged side chain.

In some embodiments, the first C-terminal amino acid and/or the second C-terminal amino acid represented by E comprise a hydrophobic side chain. For example, the first C-terminal amino acid and/or the second C-terminal amino acid represented by E comprises a hydrophobic side chain, for example a large hydrophobic side chain.

In some embodiments, w is between 3 and 1000. For example, the third amino acid represented by E comprises a large hydrophobic side chain.

In other embodiments, the peptidomimetic macrocycle as claimed excludes the sequence of:

Ac-RTQATF\$8NQWAibANle\$TNAibTR-NH₂ (SEQ ID NO: 1), Ac-RTQATF\$8NQWAibANle\$TNAibTR-NH₂ (SEQ ID NO: 2),

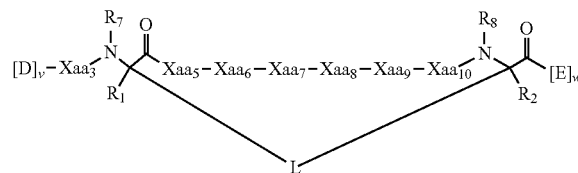
Ac-\$8SQQTF\$8LWRLAibQN-NH₂ (SEQ ID NO: 3), Ac-QSQ\$8TFSNLW\$LLAibQN-NH₂ (SEQ ID NO: 4),

Ac-QSS\$5QTF\$8NLW\$LLAibQN-NH₂ (SEQ ID NO: 5), or Ac-QSQ\$8TFSNLW\$LLAibQN-NH₂ (SEQ ID NO: 6).

In other embodiments, the peptidomimetic macrocycle as claimed excludes the sequence of:

Ac-Q\$8QQTF\$8LWRLAibQN-NH₂ (SEQ ID NO: 7).

Peptidomimetic macrocycles are also provided of the formula:



wherein:

each of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ is individually an amino acid, wherein at least three of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the corresponding position of the sequence Phe₃-X₄-His₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀-X₁₁-Ser₁₂ (SEQ ID NO: 8), where each X is an amino acid;

each D and E is independently an amino acid;

R₁ and R₂ are independently —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo-; or at least one of R₁ and R₂ forms a macrocycle-forming linker L' connected to the alpha position of one of said D or E amino acids;

each L or L' is independently a macrocycle-forming linker of the formula —L₁—L₂—;

L₁ and L₂ are independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, cycloarylene, heterocycloarylene, or [—R₄—K—R₄—]_n, each being optionally substituted with R₅;

R₃ is hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R₅;

each R₄ is alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene;

5

each K is O, S, SO, SO₂, CO, CO₂, or CONR₃;

each R₅ is independently halogen, alkyl, —OR₆, —N(R₆)₂, —SR₆, —SOR₆, —SO₂R₆, —CO₂R₆, a fluorescent moiety, a radioisotope or a therapeutic agent;

each R₆ is independently —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heterocycloalkyl, a fluorescent moiety, a radioisotope or a therapeutic agent;

R₇ is —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R₅, or part of a cyclic structure with a D residue;

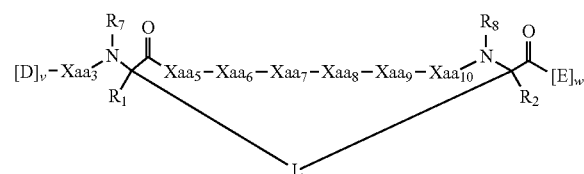
R₈ is —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R₅, or part of a cyclic structure with an E residue;

v is an integer from 1-1000, for example 1-500, 1-200, 1-100, 1-50, 1-30, 1-20, or 1-10;

w is an integer from 3-1000, for example 3-500, 3-200, 3-100, 3-50, 3-30, 3-20, or 3-10; and

n is an integer from 1-5.

In some embodiments, a peptidomimetic macrocycle has the Formula:



wherein:

each of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ is individually an amino acid, wherein at least three of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the corresponding position of the sequence Phe₃-X₄-Glu₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀/Cba₁₀-X₁₁-Ala₁₂ (SEQ ID NO: 9), where each X is an amino acid;

each D is independently an amino acid;

each E is independently an amino acid, for example an amino acid selected from Ala (alanine), D-Ala (D-alanine), Aib (α-aminoisobutyric acid), Sar (N-methyl glycine), and Ser (serine);

R₁ and R₂ are independently —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo-; or at least one of R₁ and R₂ forms a macrocycle-forming linker L' connected to the alpha position of one of said D or E amino acids;

each L or L' is independently a macrocycle-forming linker of the formula —L₁-L₂-;

L₁ and L₂ are independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, cycloarylene, heterocycloarylene, or [—R₄—K—R₄—]_n, each being optionally substituted with R₅;

R₃ is hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R₅;

each R₄ is alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene;

each K is O, S, SO, SO₂, CO, CO₂, or CONR₃;

each R₅ is independently halogen, alkyl, —OR₆, —N(R₆)₂, —SR₆, —SOR₆, —SO₂R₆, —CO₂R₆, a fluorescent moiety, a radioisotope or a therapeutic agent;

6

each R₆ is independently —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heterocycloalkyl, a fluorescent moiety, a radioisotope or a therapeutic agent;

R₇ is —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R₅, or part of a cyclic structure with a D residue;

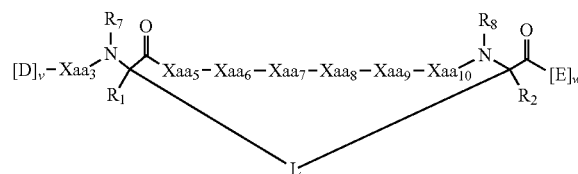
R₈ is —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R₅, or part of a cyclic structure with an E residue;

v is an integer from 1-1000, for example 1-500, 1-200, 1-100, 1-50, 1-30, 1-20, or 1-10;

w is an integer from 3-1000, for example 3-500, 3-200, 3-100, 3-50, 3-30, 3-20, or 3-10; and

n is an integer from 1-5.

In some embodiments, a peptidomimetic macrocycle has the Formula:



wherein:

each of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ is individually an amino acid, wherein at least two of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the corresponding position of the sequence Phe₃-X₄-Glu₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀/Cba₁₀-X₁₁-Ala₁₂ (SEQ ID NO: 9), where each X is an amino acid;

each D and E is independently an amino acid;

R₁ and R₂ are independently —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo-; or at least one of R₁ and R₂ forms a macrocycle-forming linker L' connected to the alpha position of one of said D or E amino acids;

each L or L' is independently a macrocycle-forming linker of the formula —L₁-L₂-, wherein L comprises at least one double bond in the E configuration;

L₁ and L₂ are independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, cycloarylene, heterocycloarylene, or [—R₄—K—R₄—]_n, each being optionally substituted with R₅;

R₃ is hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R₅;

each R₄ is alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene;

each K is O, S, SO, SO₂, CO, CO₂, or CONR₃;

each R₅ is independently halogen, alkyl, —OR₆, —N(R₆)₂, —SR₆, —SOR₆, —SO₂R₆, —CO₂R₆, a fluorescent moiety, a radioisotope or a therapeutic agent;

each R₆ is independently —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heterocycloalkyl, a fluorescent moiety, a radioisotope or a therapeutic agent;

R₇ is —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R₅, or part of a cyclic structure with a D residue;

R_8 is —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R_5 , or part of a cyclic structure with an E residue;

v is an integer from 1-1000;

w is an integer from 3-1000;

n is an integer from 1-5; and

Xaa₇ is Boc-protected tryptophan.

In some embodiments of any of the Formulas described herein, [D]_v is -Leu₁-Thr₂. In other embodiments of the Formulas described herein, each E other than the third amino acid represented by E is an amino acid selected from Ala (alanine), D-Ala (D-alanine), Aib (α -aminoisobutyric acid), Sar (N-methyl glycine), and Ser (serine).

In some embodiments, w is an integer from 3-10, for example 3-6, 3-8, 6-8, or 6-10. In some embodiments, w is 3. In other embodiments, w is 6. In some embodiments, v is an integer from 1-10, for example 2-5. In some embodiments, v is 2.

In some embodiments, peptides disclosed herein bind a binding site defined at least in part by the MDMX amino acid side chains of L17, V46, M50, Y96 (forming the rim of the pocket) and L99. Without being bound by theory, binding to such a binding site improves one or more properties such as binding affinity, induction of apoptosis, in vitro or in vivo anti-tumor efficacy, or reduced ratio of binding affinities to MDMX versus MDM2.

In some embodiments, the peptidomimetic macrocycle has improved binding affinity to MDM2 or MDMX relative to a corresponding peptidomimetic macrocycle where w is 0, 1 or 2. In other instances, the peptidomimetic macrocycle has a reduced ratio of binding affinities to MDMX versus MDM2 relative to a corresponding peptidomimetic macrocycle where w is 0, 1 or 2. In still other instances, the peptidomimetic macrocycle has improved in vitro anti-tumor efficacy against p53 positive tumor cell lines relative to a corresponding peptidomimetic macrocycle where w is 0, 1 or 2. In some embodiments, the peptidomimetic macrocycle shows improved in vitro induction of apoptosis in p53 positive tumor cell lines relative to a corresponding peptidomimetic macrocycle where w is 0, 1 or 2. In other instances, the peptidomimetic macrocycle of claim 1, wherein the peptidomimetic macrocycle has an improved in vitro anti-tumor efficacy ratio for p53 positive versus p53 negative or mutant tumor cell lines relative to a corresponding peptidomimetic macrocycle where w is 0, 1 or 2. In some instances the improved efficacy ratio in vitro, is 1-29, ≥ 30 -49, or ≥ 50 . In still other instances, the peptidomimetic macrocycle has improved in vivo anti-tumor efficacy against p53 positive tumors relative to a corresponding peptidomimetic macrocycle where w is 0, 1 or 2. In some instances the improved efficacy ratio in vivo is ≥ 29 , ≥ 30 -49, or ≥ 50 . In yet other instances, the peptidomimetic macrocycle has improved in vivo induction of apoptosis in p53 positive tumors relative to a corresponding peptidomimetic macrocycle where w is 0, 1 or 2. In some embodiments, the peptidomimetic macrocycle has improved cell permeability relative to a corresponding peptidomimetic macrocycle where w is 0, 1 or 2. In other cases, the peptidomimetic macrocycle has improved solubility relative to a corresponding peptidomimetic macrocycle where w is 0, 1 or 2.

In some embodiments, Xaa₅ is Glu or an amino acid analog thereof. In some embodiments, Xaa₅ is Glu or an amino acid analog thereof and wherein the peptidomimetic macrocycle has an improved property, such as improved binding affinity, improved solubility, improved cellular efficacy, improved cell permeability, improved in vivo or in

vitro anti-tumor efficacy, or improved induction of apoptosis relative to a corresponding peptidomimetic macrocycle where Xaa₅ is Ala.

In some embodiments, the peptidomimetic macrocycle has improved binding affinity to MDM2 or MDMX relative to a corresponding peptidomimetic macrocycle where Xaa₅ is Ala. In other embodiments, the peptidomimetic macrocycle has a reduced ratio of binding affinities to MDMX vs MDM2 relative to a corresponding peptidomimetic macrocycle where Xaa₅ is Ala. In some embodiments, the peptidomimetic macrocycle has improved solubility relative to a corresponding peptidomimetic macrocycle where Xaa₅ is Ala, or the peptidomimetic macrocycle has improved cellular efficacy relative to a corresponding peptidomimetic macrocycle where Xaa₅ is Ala.

In some embodiments, Xaa₅ is Glu or an amino acid analog thereof and wherein the peptidomimetic macrocycle has improved biological activity, such as improved binding affinity, improved solubility, improved cellular efficacy, improved helicity, improved cell permeability, improved in vivo or in vitro anti-tumor efficacy, or improved induction of apoptosis relative to a corresponding peptidomimetic macrocycle where Xaa₅ is Ala.

In some embodiments, the peptidomimetic macrocycle has an activity against a p53+/+ cell line which is at least 2-fold, 3-fold, 5-fold, 10-fold, 20-fold, 30-fold, 50-fold, 70-fold, or 100-fold greater than its binding affinity against a p53-/- cell line. In some embodiments, the peptidomimetic macrocycle has an activity against a p53+/+ cell line which is between 1 and 29-fold, between 30 and 49-fold, or ≥ 50 -fold greater than its binding affinity against a p53-/- cell line. Activity can be measured, for example, as an IC50 value. For example, the p53+/+ cell line is SJSA-1, RKO, HCT-116, or MCF-7 and the p53-/- cell line is RKO-E6 or SW-480. In some embodiments, the peptide has an IC50 against the p53+/+ cell line of less than 1 μ M.

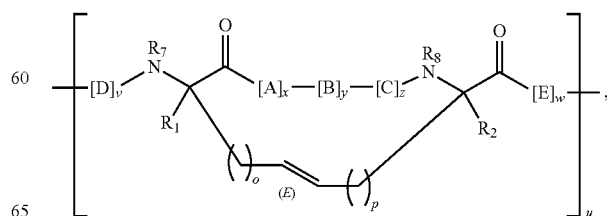
In some embodiments, Xaa₅ is Glu or an amino acid analog thereof and the peptidomimetic macrocycle has an activity against a p53+/+ cell line which is at least 10-fold greater than its binding affinity against a p53-/- cell line.

Additionally, a method is provided of treating cancer in a subject comprising administering to the subject a peptidomimetic macrocycle. In some embodiments, the cancer is head and neck cancer, melanoma, lung cancer, breast cancer, or glioma.

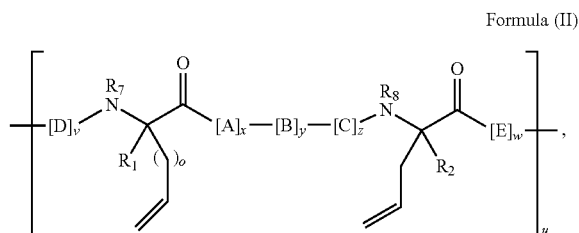
Also provided is a method of modulating the activity of p53 or MDM2 or MDMX in a subject comprising administering to the subject a peptidomimetic macrocycle, or a method of antagonizing the interaction between p53 and MDM2 and/or MDMX proteins in a subject comprising administering to the subject such a peptidomimetic macrocycle.

Provided herein is a method of preparing a composition comprising a peptidomimetic macrocycle of Formula (I):

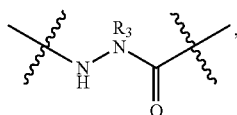
Formula (I)



comprising an amino acid sequence which is about 60% to about 100% identical to an amino acid sequence selected from the group consisting of the amino acid sequences in Table 1, Table 1a, Table 1b, or Table 1c, the method comprising treating a compound of Formula (II)



with a catalyst to result in the compound of Formula I wherein in the compound(s) of Formulae (I) and (II) each A, C, D, and E is independently an amino acid; each B is independently an amino acid,



[—NH—L₃—CO—], [—NH—L₃—SO₂—], or [—NH—L₃—];

each R₁ and R₂ are independently hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halogen; or at least one of R₁ and R₂ forms a macrocycle-forming linker L' connected to the alpha position of one of the D or E amino acids;

each R₃ is independently hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R₅;

each L' is independently a macrocycle-forming linker of the formula —L₁—L₂—;

each L₁, L₂ and L₃ are independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, cycloarylene, heterocycloarylene, or [—R₄—K—R₄—]_n, each being optionally substituted with R₅;

each R₄ and R₄' is independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene;

each K is independently O, S, SO, SO₂, CO, CO₂, or CONR₃;

each R₅ is independently hydrogen, alkyl, —OR₆, —N(R₆)₂, —SR₆, —SOR₆, —SO₂R₆, —CO₂R₆, a fluorescent moiety, a radioisotope or a therapeutic agent;

each R₆ is independently hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heterocycloalkyl, a fluorescent moiety, a radioisotope or a therapeutic agent;

each R₇ is independently hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R₅, or part of a cyclic structure with a D residue;

each R₈ is independently hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, het-

erocycloalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R₅, or part of a cyclic structure with an E residue;

each v and w are independently integers from 1-1000;

u is an integer from 1-10;

each x, y and z are independently integers from 0-10;

each n is independently an integer from 1-5;

each o is independently an integer from 1 to 15;

each p is independently an integer from 1 to 15;

“(E)” indicates a trans double bond; and

one or more of the amino acids A, C and/or B when B is an amino acid, present in the compounds of Formulae (I) and (II), has a side chain bearing a protecting group.

In some embodiments, the protecting group is a nitrogen atom protecting group.

In some embodiments, the protecting group is a Boc group.

In some embodiments, the side chain of the amino acid bearing the protecting group comprises a protected indole.

In some embodiments, the amino acid bearing the protecting group on its side chain is tryptophan (W) that is protected by the protecting group on its indole nitrogen. For example, the protecting group is a Boc group.

In some embodiments, after the step of contacting the compound of Formula II with catalyst the compound of Formula (I) is obtained in equal or higher amounts than a corresponding compound which is a Z isomer. For example, after the step of contacting the compound of Formula II with catalyst the compound of Formula (I) is obtained in a 2, 3, 4, 5, 6, 7, 8, 9, or 10-fold higher amount than the corresponding compound which is a Z isomer.

In some embodiments, the catalyst is a ruthenium catalyst.

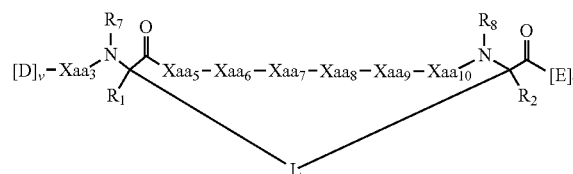
In some embodiments, the method further comprises the step of treating the compounds of Formula (I) with a reducing agent or an oxidizing agent.

In some embodiments, the compound of Formula (II) is attached to a solid support. In other embodiments, the compound of Formula (II) is not attached to a solid support.

In some embodiments, the method further comprises removing the protecting group(s) from the compounds of Formula (I).

In some embodiments, the ring closing metathesis is conducted at a temperature ranging from about 20° C. to about 80° C.

In some embodiments, the peptidomimetic macrocycle of Formula (I) has the Formula:



wherein:

each of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ is individually an amino acid, wherein at least two of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the corresponding position of the sequence Phe₃-X₄-His₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀-X₁₁-Ser₁₂ (SEQ ID NO: 8), where each X is an amino acid; each D and E is independently an amino acid;

R₁ and R₂ are independently —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo-; or at

11

least one of R_1 and R_2 forms a macrocycle-forming linker L' connected to the alpha position of one of said D or E amino acids;

each L or L' is independently a macrocycle-forming linker of the formula $-L_1-L_2-$, wherein L comprises at least one double bond in the E configuration;

L_1 and L_2 are independently alkylene, alkenylene, alkenylene, heteroalkylene, cycloalkylene, heterocycloalkylene, cycloarylene, heterocycloarylene, or $[-R_4-K-R_4-]_m$, each being optionally substituted with R_5 ;

R_3 is hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R_5 ;

each R_4 is alkylene, alkenylene, alkenylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene;

each K is O, S, SO, SO_2 , CO, CO_2 , or $CONR_3$;

each R_5 is independently halogen, alkyl, $-OR_6$, $-N(R_6)_2$, $-SR_6$, $-SOR_6$, $-SO_2R_6$, $-CO_2R_6$, a fluorescent moiety, a radioisotope or a therapeutic agent;

each R_6 is independently $-H$, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heterocycloalkyl, a fluorescent moiety, a radioisotope or a therapeutic agent;

R_7 is $-H$, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R_5 , or part of a cyclic structure with a D residue;

R_8 is $-H$, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R_5 , or part of a cyclic structure with an E residue;

v is an integer from 1-1000;

w is an integer from 3-1000;

n is an integer from 1-5; and

Xaa_7 is Boc-protected tryptophan.

In some embodiments, the peptidomimetic macrocycle of Formula (I) comprises an α -helix.

INCORPORATION BY REFERENCE

All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

FIG. 1 shows a structure of peptidomimetic macrocycle 46 (Table 2b), a p53 peptidomimetic macrocycle, complexed with MDMX (Primary SwissProt accession number Q7ZUW7; Entry MDM4_DANRE).

FIG. 2 shows overlaid structures of p53 peptidomimetic macrocycles 142 (Table 2b) and SP43 bound to MDMX (Primary SwissProt accession number Q7ZUW7; Entry MDM4_DANRE).

FIG. 3 shows the effect of SP154, a peptidomimetic macrocycle, on tumor growth in a mouse MCF-7 xenograft model.

12

FIG. 4 shows the effect of SP249, a peptidomimetic macrocycle, on tumor growth in a mouse MCF-7 xenograft model.

FIG. 5 shows the effect of SP315, a peptidomimetic macrocycle, on tumor growth in a mouse MCF-7 xenograft model.

FIG. 6 shows the effect of SP252, a point mutation of SP154, on tumor growth in a mouse MCF-7 xenograft model.

FIG. 7 shows a plot of solubility for peptidomimetic macrocycles with varying C-terminal extensions.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the term "macrocycle" refers to a molecule having a chemical structure including a ring or cycle formed by at least 9 covalently bonded atoms.

As used herein, the term "peptidomimetic macrocycle" or "crosslinked polypeptide" refers to a compound comprising a plurality of amino acid residues joined by a plurality of peptide bonds and at least one macrocycle-forming linker which forms a macrocycle between a first naturally-occurring or non-naturally-occurring amino acid residue (or analog) and a second naturally-occurring or non-naturally-occurring amino acid residue (or analog) within the same molecule. Peptidomimetic macrocycle include embodiments where the macrocycle-forming linker connects the α carbon of the first amino acid residue (or analog) to the α carbon of the second amino acid residue (or analog). The peptidomimetic macrocycles optionally include one or more non-peptide bonds between one or more amino acid residues and/or amino acid analog residues, and optionally include one or more non-naturally-occurring amino acid residues or amino acid analog residues in addition to any which form the macrocycle. A "corresponding uncrosslinked polypeptide" when referred to in the context of a peptidomimetic macrocycle is understood to relate to a polypeptide of the same length as the macrocycle and comprising the equivalent natural amino acids of the wild-type sequence corresponding to the macrocycle.

As used herein, the term "stability" refers to the maintenance of a defined secondary structure in solution by a peptidomimetic macrocycle as measured by circular dichroism, NMR or another biophysical measure, or resistance to proteolytic degradation in vitro or in vivo. Non-limiting examples of secondary structures contemplated herein are α -helices, 3_{10} helices, β -turns, and β -pleated sheets.

As used herein, the term "helical stability" refers to the maintenance of α helical structure by a peptidomimetic macrocycle as measured by circular dichroism or NMR. For example, in some embodiments, a peptidomimetic macrocycle exhibits at least a 1.25, 1.5, 1.75 or 2-fold increase in α -helicity as determined by circular dichroism compared to a corresponding uncrosslinked macrocycle.

The term "amino acid" refers to a molecule containing both an amino group and a carboxyl group. Suitable amino acids include, without limitation, both the D- and L-isomers of the naturally-occurring amino acids, as well as non-naturally occurring amino acids prepared by organic synthesis or other metabolic routes. The term amino acid, as used herein, includes, without limitation, α -amino acids, natural amino acids, non-natural amino acids, and amino acid analogs.

The term " α -amino acid" refers to a molecule containing both an amino group and a carboxyl group bound to a carbon which is designated the α -carbon.

13

The term “ β -amino acid” refers to a molecule containing both an amino group and a carboxyl group in a 3 configuration.

The term “naturally occurring amino acid” refers to any one of the twenty amino acids commonly found in peptides synthesized in nature, and known by the one letter abbreviations A, R, N, C, D, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y and V.

The following table shows a summary of the properties of natural amino acids:

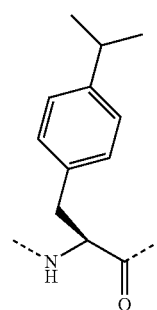
Amino Acid	3-Letter Code	1-Letter Code	Side-chain Polarity	Side-chain charge (pH 7.4)	Hydropathy Index
Alanine	Ala	A	nonpolar	neutral	1.8
Arginine	Arg	R	polar	positive	-4.5
Asparagine	Asn	N	polar	neutral	-3.5
Aspartic acid	Asp	D	polar	negative	-3.5
Cysteine	Cys	C	polar	neutral	2.5
Glutamic acid	Glu	E	polar	negative	-3.5
Glutamine	Gln	Q	polar	neutral	-3.5
Glycine	Gly	G	nonpolar	neutral	-0.4
Histidine	His	H	polar	positive(10%) neutral(90%)	-3.2
Isoleucine	Ile	I	nonpolar	neutral	4.5
Leucine	Leu	L	nonpolar	neutral	3.8
Lysine	Lys	K	polar	positive	-3.9
Methionine	Met	M	nonpolar	neutral	1.9
Phenylalanine	Phe	F	nonpolar	neutral	2.8
Proline	Pro	P	nonpolar	neutral	-1.6
Serine	Ser	S	polar	neutral	-0.8
Threonine	Thr	T	polar	neutral	-0.7
Tryptophan	Trp	W	nonpolar	neutral	-0.9
Tyrosine	Tyr	Y	polar	neutral	-1.3
Valine	Val	V	nonpolar	neutral	4.2

“Hydrophobic amino acids” include small hydrophobic amino acids and large hydrophobic amino acids. “Small hydrophobic amino acid” are glycine, alanine, proline, and analogs thereof. “Large hydrophobic amino acids” are valine, leucine, isoleucine, phenylalanine, methionine, tryptophan, and analogs thereof. “Polar amino acids” are serine, threonine, asparagine, glutamine, cysteine, tyrosine, and analogs thereof. “Charged amino acids” are lysine, arginine, histidine, aspartate, glutamate, and analogs thereof.

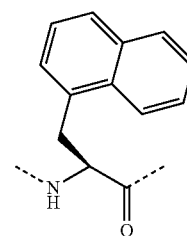
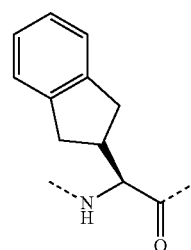
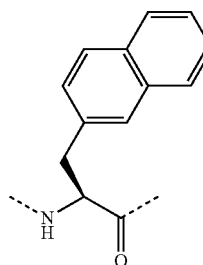
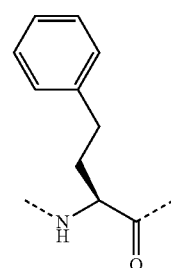
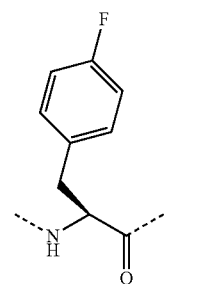
The term “amino acid analog” refers to a molecule which is structurally similar to an amino acid and which can be substituted for an amino acid in the formation of a peptidomimetic macrocycle. Amino acid analogs include, without limitation, β -amino acids and amino acids where the amino or carboxy group is substituted by a similarly reactive group (e.g., substitution of the primary amine with a secondary or tertiary amine, or substitution of the carboxy group with an ester).

The term “non-natural amino acid” refers to an amino acid which is not one of the twenty amino acids commonly found in peptides synthesized in nature, and known by the one letter abbreviations A, R, N, C, D, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y and V. Non-natural amino acids or amino acid analogs include, without limitation, structures according to the following:

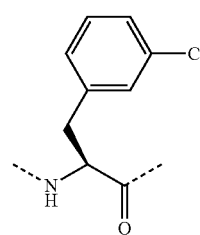
14



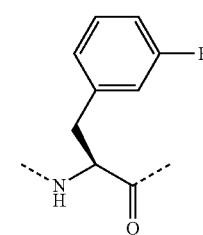
F4tBu

1-Naphtalanine
(1Nal)Indanyl glycine
(Igl)2-Naphtalanine
(2Nal)homophenylalanine
(hF)

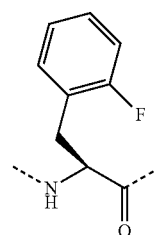
F4F



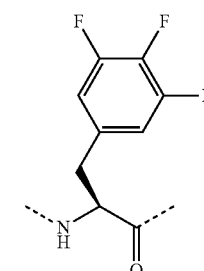
F3Cl = 3cf



F3F = 3ff



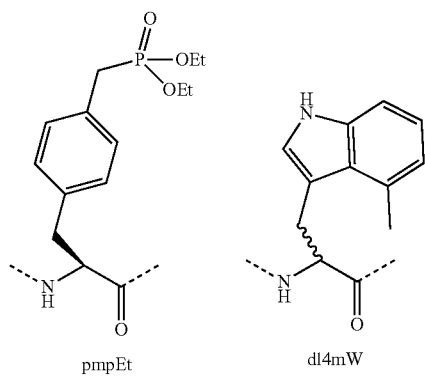
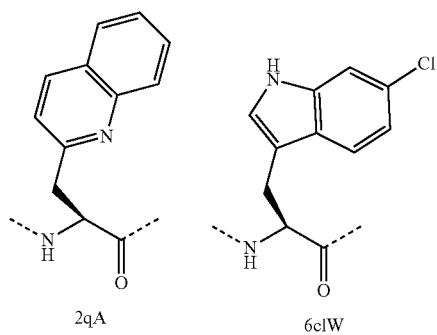
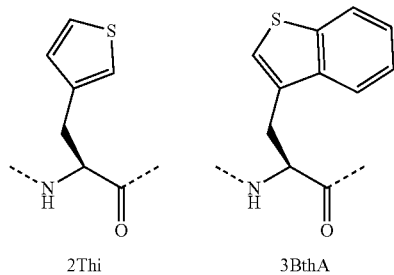
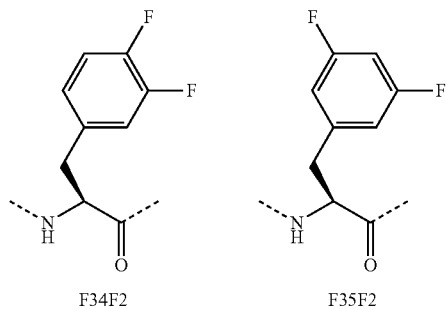
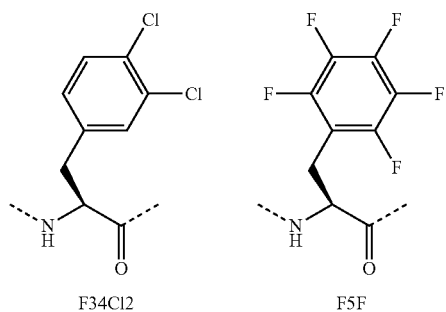
F2F = 2ff



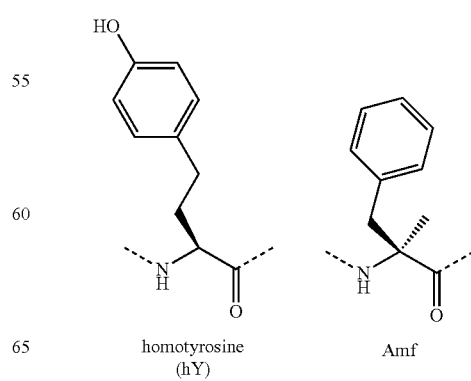
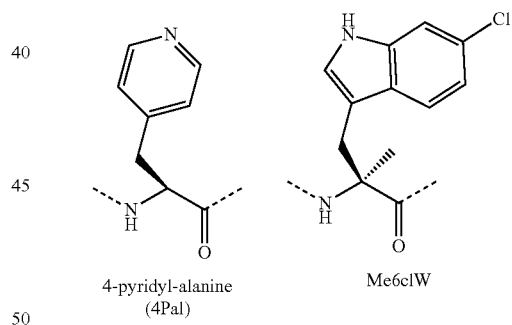
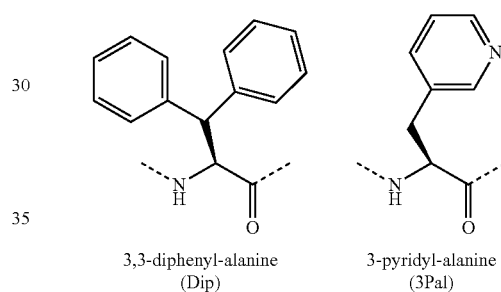
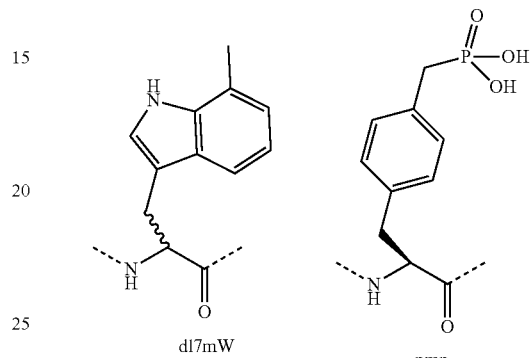
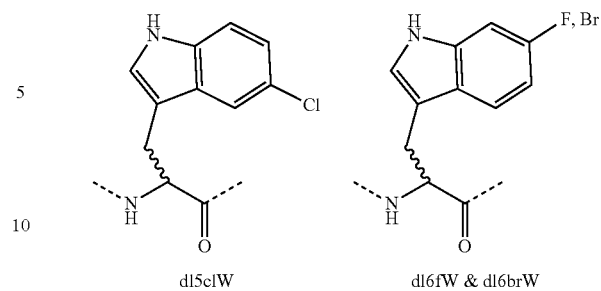
F345F3

15

-continued

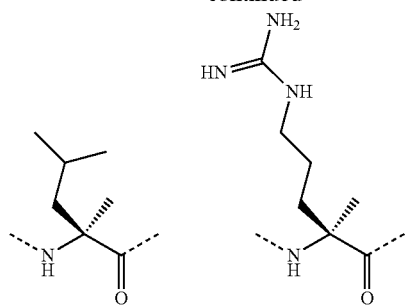
**16**

-continued



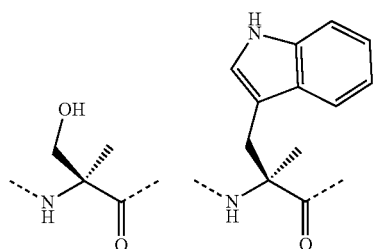
17

-continued



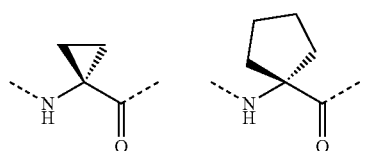
Aml

Amr



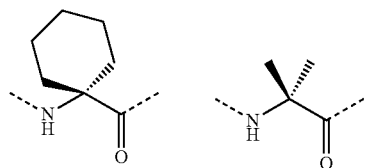
Ams

Amw



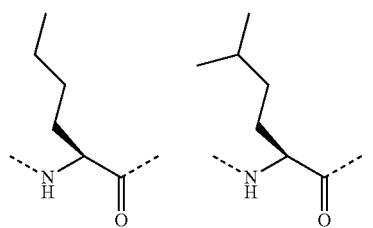
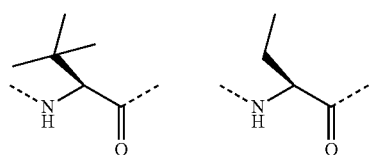
Ac3c

Ac5c



Ac6c

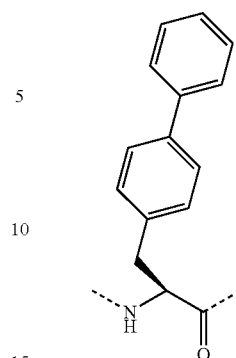
Aib

Norleucine
(Nle)Homoleucine
(hL)tert-Butyl glycine
(Tle)

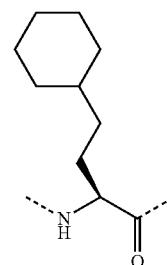
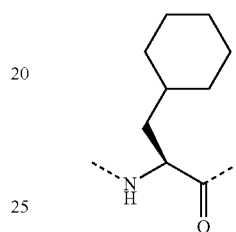
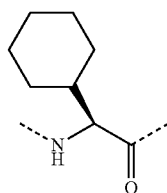
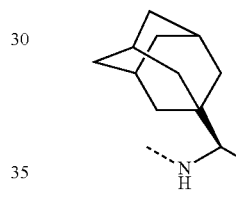
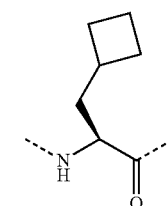
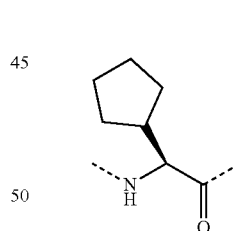
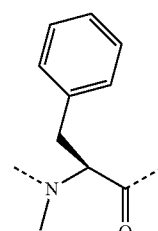
Abu

18

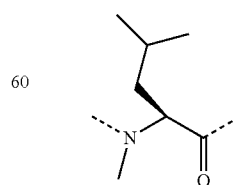
-continued



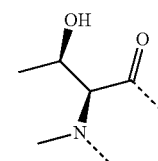
Bip

homocyclohexyl alanine
(hCha)cyclohexyl alanine
(Cha)cyclohexyl glycine
(Chg)Adamantyl glycine
(Adm)cyclobutyl alanine
(Cba)cyclopentyl glycine
(Cpg)

NmF



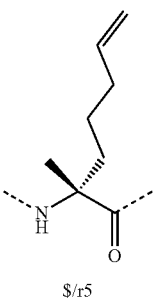
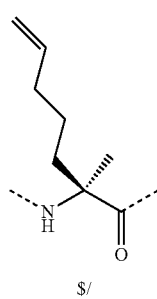
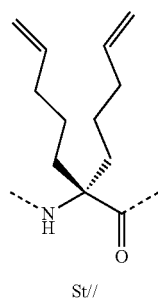
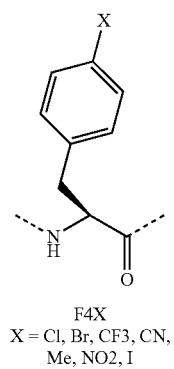
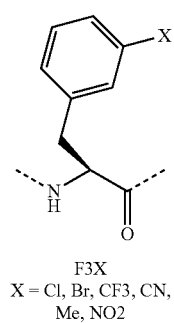
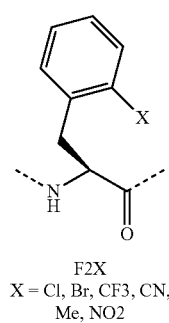
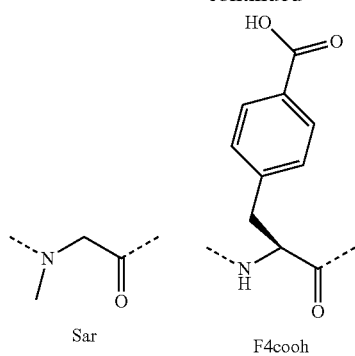
NmL



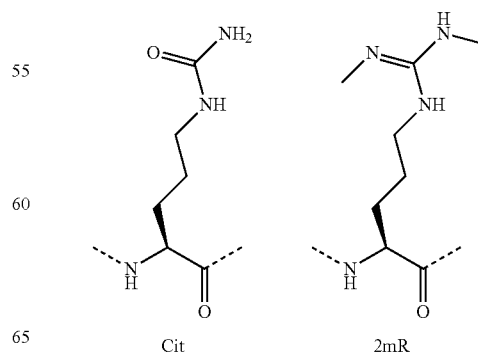
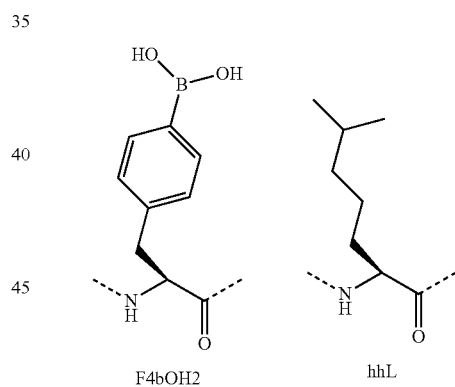
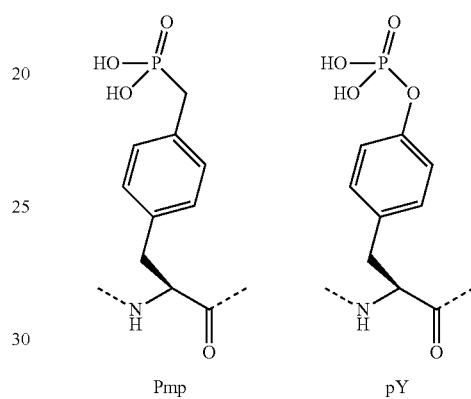
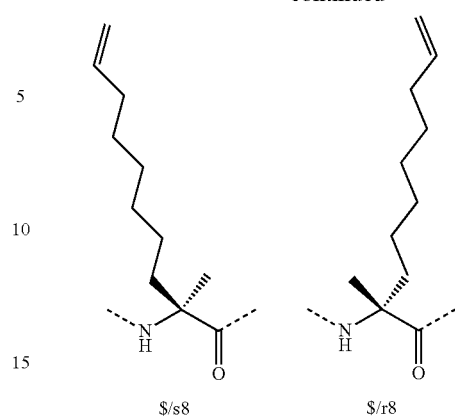
NmT

19

-continued

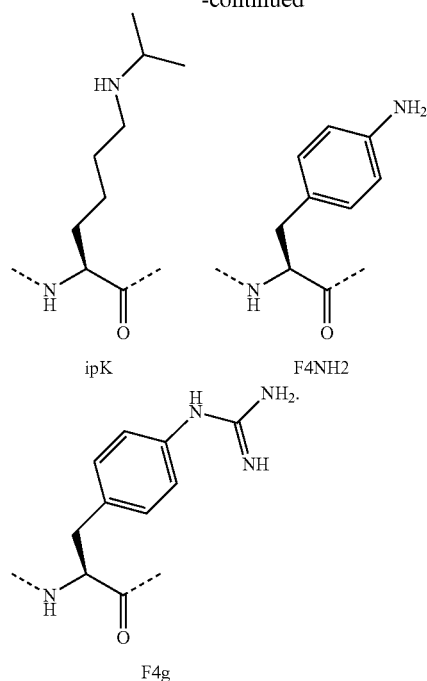
**20**

-continued



21

-continued



Amino acid analogs include β -amino acid analogs. Examples of β -amino acid analogs include, but are not limited to, the following: cyclic β -amino acid analogs; β -alanine; (R)- β -phenylalanine; (R)-1,2,3,4-tetrahydro-isoquinoline-3-acetic acid; (R)-3-amino-4-(1-naphthyl)-butyric acid; (R)-3-amino-4-(2,4-dichlorophenyl)butyric acid; (R)-3-amino-4-(2-chlorophenyl)-butyric acid; (R)-3-amino-4-(2-cyanophenyl)-butyric acid; (R)-3-amino-4-(2-fluorophenyl)-butyric acid; (R)-3-amino-4-(2-furyl)-butyric acid; (R)-3-amino-4-(2-methylphenyl)-butyric acid; (R)-3-amino-4-(2-naphthyl)-butyric acid; (R)-3-amino-4-(2-thienyl)-butyric acid; (R)-3-amino-4-(2-trifluoromethylphenyl)-butyric acid; (R)-3-amino-4-(3,4-dichlorophenyl)butyric acid; (R)-3-amino-4-(3,4-difluorophenyl)butyric acid; (R)-3-amino-4-(3-benzothienyl)-butyric acid; (R)-3-amino-4-(3-chlorophenyl)-butyric acid; (R)-3-amino-4-(3-cyanophenyl)-butyric acid; (R)-3-amino-4-(3-fluorophenyl)-butyric acid; (R)-3-amino-4-(3-pyridyl)-butyric acid; (R)-3-amino-4-(3-thienyl)-butyric acid; (R)-3-amino-4-(3-trifluoromethylphenyl)-butyric acid; (R)-3-amino-4-(4-bromophenyl)-butyric acid; (R)-3-amino-4-(4-chlorophenyl)-butyric acid; (R)-3-amino-4-(4-cyanophenyl)-butyric acid; (R)-3-amino-4-(4-fluorophenyl)-butyric acid; (R)-3-amino-4-(4-iodophenyl)-butyric acid; (R)-3-amino-4-(4-methylphenyl)-butyric acid; (R)-3-amino-4-(4-nitrophenyl)-butyric acid; (R)-3-amino-4-(4-pyridyl)-butyric acid; (R)-3-amino-4-(4-trifluoromethylphenyl)-butyric acid; (R)-3-amino-4-pentafluoro-phenylbutyric acid; (R)-3-amino-5-hexenoic acid; (R)-3-amino-5-hexynoic acid; (R)-3-amino-5-phenylpentanoic acid; (R)-3-amino-6-phenyl-5-hexenoic acid; (S)-1,2,3,4-tetrahydroisoquinoline-3-acetic acid; (S)-3-amino-4-(1-naphthyl)-butyric acid; (S)-3-amino-4-(2,4-dichlorophenyl)butyric acid; (S)-3-amino-4-(2-chlorophenyl)-butyric acid; (S)-3-amino-4-(2-cyanophenyl)-butyric acid; (S)-3-amino-4-(2-fluorophenyl)-butyric acid; (S)-3-amino-4-(2-furyl)-butyric acid; (S)-3-amino-4-(2-methylphenyl)-butyric acid; (S)-3-amino-4-(2-naphthyl)-butyric acid; (S)-3-amino-4-(2-thienyl)-butyric acid; (S)-3-amino-4-(2-trifluoromethylphenyl)-

22

butyric acid; (S)-3-amino-4-(3,4-dichlorophenyl)butyric acid; (S)-3-amino-4-(3,4-difluorophenyl)butyric acid; (S)-3-amino-4-(3-benzothienyl)-butyric acid; (S)-3-amino-4-(3-chlorophenyl)-butyric acid; (S)-3-amino-4-(3-cyanophenyl)-butyric acid; (S)-3-amino-4-(3-fluorophenyl)-butyric acid; (S)-3-amino-4-(3-methylphenyl)-butyric acid; (S)-3-amino-4-(3-pyridyl)-butyric acid; (S)-3-amino-4-(3-thienyl)-butyric acid; (S)-3-amino-4-(3-trifluoromethylphenyl)-butyric acid; (S)-3-amino-4-(4-bromophenyl)-butyric acid; (S)-3-amino-4-(4-chlorophenyl)-butyric acid; (S)-3-amino-4-(4-cyanophenyl)-butyric acid; (S)-3-amino-4-(4-fluorophenyl)-butyric acid; (S)-3-amino-4-(4-iodophenyl)-butyric acid; (S)-3-amino-4-(4-methylphenyl)-butyric acid; (S)-3-amino-4-(4-nitrophenyl)-butyric acid; (S)-3-amino-4-(4-pyridyl)-butyric acid; (S)-3-amino-4-(4-trifluoromethylphenyl)-butyric acid; (S)-3-amino-4-pentafluoro-phenylbutyric acid; (S)-3-amino-5-hexenoic acid; (S)-3-amino-5-hexynoic acid; (S)-3-amino-5-phenylpentanoic acid; (S)-3-amino-6-phenyl-5-hexenoic acid; 1,2,5,6-tetrahydropyridine-3-carboxylic acid; 1,2,5,6-tetrahydropyridine-4-carboxylic acid; 3-amino-3-(2-chlorophenyl)-propionic acid; 3-amino-3-(2-thienyl)-propionic acid; 3-amino-3-(3-bromophenyl)-propionic acid; 3-amino-3-(4-chlorophenyl)-propionic acid; 3-amino-3-(4-methoxyphenyl)-propionic acid; 3-amino-4,4,4-trifluoro-butylbutyric acid; 3-aminoadipic acid; D- β -phenylalanine; β -leucine; L- β -homoalanine; L- β -homoaspartic acid γ -benzyl ester; L- β -homoglutamic acid δ -benzyl ester; L- β -homoisoleucine; L- β -homoleucine; L- β -homomethionine; L- β -homophenylalanine; L- β -homoproline; L- β -homotryptophan; L- β -homovaline; L-N ω -benzyloxycarbonyl- β -homolysine; N ω -L- β -homoarginine; O-benzyl-L- β -homohydroxyproline; O-benzyl-L- β -homoserine; O-benzyl-L- β -homothreonine; O-benzyl-L- β -homotyrosine; γ -trityl-L- β -homoasparagine; (R)- β -phenylalanine; L- β -homoaspartic acid γ -t-butyl ester; L- β -homoglutamic acid δ -t-butyl ester; L-N ω - β -homolysine; N δ -trityl-L- β -homoglutamine; N ω -2,2,4,6,7-pentamethyl-dihydrobenzofuran-5-sulfonyl-L- β -homoarginine; O-t-butyl-L- β -homohydroxy-proline; O-t-butyl-L- β -homoserine; O-t-butyl-L- β -homothreonine; O-t-butyl-L- β -homotyrosine; 2-aminocyclopentane carboxylic acid; and 2-aminocyclohexane carboxylic acid.

Amino acid analogs include analogs of alanine, valine, glycine or leucine. Examples of amino acid analogs of alanine, valine, glycine, and leucine include, but are not limited to, the following: α -methoxyglycine; α -allyl-L-alanine; α -aminoisobutyric acid; α -methyl-leucine; β -(1-naphthyl)-D-alanine; β -(1-naphthyl)-L-alanine; β -(2-naphthyl)-D-alanine; β -(2-naphthyl)-L-alanine; β -(2-pyridyl)-D-alanine; β -(2-pyridyl)-L-alanine; β -(2-thienyl)-D-alanine; β -(2-thienyl)-L-alanine; β -(3-benzothienyl)-D-alanine; β -(3-benzothienyl)-L-alanine; β -(3-pyridyl)-D-alanine; β -(3-pyridyl)-L-alanine; β -(4-pyridyl)-D-alanine; β -(4-pyridyl)-L-alanine; β -chloro-L-alanine; β -cyano-L-alanine; β -cyclohexyl-D-alanine; β -cyclohexyl-L-alanine; β -cyclopenten-1-yl-alanine; β -cyclopentyl-alanine; β -cyclopropyl-L-Ala-OH.dicyclohexylammonium salt; β -t-butyl-D-alanine; β -t-butyl-L-alanine; γ -aminobutyric acid; L- α , β -diaminopropionic acid; 2,4-dinitro-phenylglycine; 2,5-dihydro-D-phenylglycine; 2-amino-4,4,4-trifluorobutyric acid; 2-fluoro-phenylglycine; 3-amino-4,4,4-trifluorobutyric acid; 3-fluoro-valine; 4,4,4-trifluoro-valine; 4,5-dehydro-L-leu-OH.dicyclohexylammonium salt; 4-fluoro-D-phenylglycine; 4-fluoro-L-phenylglycine; 4-hydroxy-D-phenylglycine; 5,5,5-trifluoro-leucine; 6-aminohexanoic acid; cyclopentyl-D-Gly-OH.dicyclohexylammonium salt; cyclopentyl-Gly-OH.dicyclohexylammonium salt; D- α , β -diaminopropionic acid; D- α -aminobutyric acid; D- α -t-

butylglycine; D-(2-thienyl)glycine; D- β -thienylglycine; D-2-aminocaproic acid; D-2-indanylglycine; D-allylglycine.dicyclohexylammonium salt; D-cyclohexylglycine; D-norvaline; D-phenylglycine; β -aminobutyric acid; β -aminoisobutyric acid; (2-bromophenyl)glycine; (2-methoxyphenyl)glycine; (2-methylphenyl)glycine; (2-thiazoyl)glycine; (2-thienyl)glycine; 2-amino-3-(dimethylamino)-propionic acid; L- α , β -diaminopropionic acid; L- α -aminobutyric acid; L- α -t-butylglycine; L- β -thienylglycine; L-2-amino-3-(dimethylamino)-propionic acid; L-2-aminocaproic acid dicyclohexyl-ammonium salt; L-2-indanylglycine; L-allylglycine.dicyclohexyl ammonium salt; L-cyclohexylglycine; L-phenylglycine; L-propargylglycine; L-norvaline; N- α -aminomethyl-L-alanine; D- α , γ -diaminobutyric acid; L- α , γ -diaminobutyric acid; β -cyclopropyl-L-alanine; (N- β -(2,4-dinitrophenyl))-L- α , β -diaminopropionic acid; (N- β -1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl)-D- α , β -diaminopropionic acid; (N- β -1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl)-L- α , β -diaminopropionic acid; (N- β -4-methyltrityl)-L- α , β -diaminopropionic acid; (N- β -allyloxycarbonyl)-L- α , β -diaminopropionic acid; (N- γ -1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl)-D- α , γ -diaminobutyric acid; (N- γ -1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl)-L- α , γ -diaminobutyric acid; (N- γ -4-methyltrityl)-D- α , γ -diaminobutyric acid; (N- γ -4-methyltrityl)-L- α , γ -diaminobutyric acid; (N- γ -allyloxycarbonyl)-L- α , γ -diaminobutyric acid; D- α , γ -diaminobutyric acid; 4,5-dehydro-L-leucine; cyclopentyl-D-Gly-OH; cyclopentyl-Gly-OH; D-allylglycine; D-homocyclohexylalanine; L-1-pyrenylalanine; L-2-aminocaproic acid; L-allylglycine; L-homocyclohexylalanine; and N-(2-hydroxy-4-methoxy-Bzl)-Gly-OH.

Amino acid analogs include analogs of arginine or lysine. Examples of amino acid analogs of arginine and lysine include, but are not limited to, the following: citrulline; L-2-amino-3-guanidinopropionic acid; L-2-amino-3-ureidopropionic acid; L-citrulline; Lys(Me)₂-OH; Lys(N₃)-OH; N δ -benzyloxycarbonyl-L-ornithine; N ω -nitro-D-arginine; N ω -nitro-L-arginine; α -methyl-ornithine; 2,6-diaminoheptanedioic acid; L-ornithine; (N δ -1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl)-D-ornithine; (N δ -1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl)-L-ornithine; (N δ -4-methyltrityl)-D-ornithine; (N δ -4-methyltrityl)-L-ornithine; D-ornithine; L-ornithine; Arg(Me)(Pbf)-OH; Arg(Me)₂-OH (asymmetrical); Arg(Me)₂-OH (symmetrical); Lys(ivDde)-OH; Lys(Me)₂-OH.HCl; Lys(Me₃)-OH chloride; N ω -nitro-D-arginine; and N ω -nitro-L-arginine.

Amino acid analogs include analogs of aspartic or glutamic acids. Examples of amino acid analogs of aspartic and glutamic acids include, but are not limited to, the following: α -methyl-D-aspartic acid; α -methyl-glutamic acid; α -methyl-L-aspartic acid; γ -methylene-glutamic acid; (N-ethyl)-L-glutamine; [N- α -(4-aminobenzoyl)]-L-glutamic acid; 2,6-diaminopimelic acid; L- α -aminosuberlic acid; D-2-aminoadipic acid; D- α -aminosuberlic acid; α -aminopimelic acid; iminodiacetic acid; L-2-aminoadipic acid; threo- β -methyl-aspartic acid; γ -carboxy-D-glutamic acid γ , γ -di-t-butyl ester; γ -carboxy-L-glutamic acid γ , γ -di-t-butyl ester; Glu(OAll)-OH; L-Asu(OtBu)-OH; and pyroglutamic acid.

Amino acid analogs include analogs of cysteine and methionine. Examples of amino acid analogs of cysteine and methionine include, but are not limited to, Cys(farnesyl)-OH, Cys(farnesyl)-OMe, α -methyl-methionine, Cys(2-hydroxyethyl)-OH, Cys(3-aminopropyl)-OH, 2-amino-4-(ethylthio)butyric acid, buthionine, buthioninesulfoximine, ethionine, methionine methylsulfonium chloride, selenomethionine, cysteic acid, [2-(4-pyridyl)ethyl]-DL-penicil-

lamine, [2-(4-pyridyl)ethyl]-L-cysteine, 4-methoxybenzyl-D-penicillamine, 4-methoxybenzyl-L-penicillamine, 4-methylbenzyl-D-penicillamine, 4-methylbenzyl-L-penicillamine, benzyl-D-cysteine, benzyl-L-cysteine, benzyl-DL-homocysteine, carbamoyl-L-cysteine, carboxyethyl-L-cysteine, carboxymethyl-L-cysteine, diphenylmethyl-L-cysteine, ethyl-L-cysteine, methyl-L-cysteine, t-butyl-D-cysteine, trityl-L-homocysteine, trityl-D-penicillamine, cystathionine, homocystine, L-homocystine, (2-aminoethyl)-L-cysteine, seleno-L-cystine, cystathionine, Cys (StBu)-OH, and acetamidomethyl-D-penicillamine.

Amino acid analogs include analogs of phenylalanine and tyrosine. Examples of amino acid analogs of phenylalanine and tyrosine include β -methyl-phenylalanine, β -hydroxy-phenylalanine, α -methyl-3-methoxy-DL-phenylalanine, α -methyl-D-phenylalanine, α -methyl-L-phenylalanine, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, 2,4-dichlorophenylalanine, 2-(trifluoromethyl)-D-phenylalanine, 2-(trifluoromethyl)-L-phenylalanine, 2-bromo-D-phenylalanine, 2-bromo-L-phenylalanine, 2-chloro-D-phenylalanine, 2-chloro-L-phenylalanine, 2-cyano-D-phenylalanine, 2-cyano-L-phenylalanine, 2-fluoro-D-phenylalanine, 2-fluoro-L-phenylalanine, 2-methyl-D-phenylalanine, 2-methyl-L-phenylalanine, 2-nitro-D-phenylalanine, 2-nitro-L-phenylalanine, 2,4;5-trihydroxy-phenylalanine, 3,4,5-trifluoro-D-phenylalanine, 3,4,5-trifluoro-L-phenylalanine, 3,4-dichloro-D-phenylalanine, 3,4-dichloro-L-phenylalanine, 3,4-difluoro-D-phenylalanine, 3,4-difluoro-L-phenylalanine, 3,4-dihydroxy-L-phenylalanine, 3,4-dimethoxy-L-phenylalanine, 3,5,3'-triiodo-L-thyronine, 3,5-diiodo-D-tyrosine, 3,5-diiodo-L-tyrosine, 3,5-diiodo-L-thyronine, 3-(trifluoromethyl)-D-phenylalanine, 3-(trifluoromethyl)-L-phenylalanine, 3-amino-L-tyrosine, 3-bromo-D-phenylalanine, 3-bromo-L-phenylalanine, 3-chloro-D-phenylalanine, 3-chloro-L-phenylalanine, 3-chloro-L-tyrosine, 3-cyano-D-phenylalanine, 3-cyano-L-phenylalanine, 3-fluoro-D-phenylalanine, 3-fluoro-L-phenylalanine, 3-fluoro-tyrosine, 3-iodo-D-phenylalanine, 3-iodo-L-phenylalanine, 3-iodo-L-tyrosine, 3-methoxy-L-tyrosine, 3-methyl-D-phenylalanine, 3-methyl-L-phenylalanine, 3-nitro-D-phenylalanine, 3-nitro-L-phenylalanine, 3-nitro-L-tyrosine, 4-(trifluoromethyl)-D-phenylalanine, 4-(trifluoromethyl)-L-phenylalanine, 4-amino-D-phenylalanine, 4-amino-L-phenylalanine, 4-benzoyl-D-phenylalanine, 4-benzoyl-L-phenylalanine, 4-bis(2-chloroethyl)amino-L-phenylalanine, 4-bromo-D-phenylalanine, 4-bromo-L-phenylalanine, 4-chloro-D-phenylalanine, 4-chloro-L-phenylalanine, 4-cyano-D-phenylalanine, 4-cyano-L-phenylalanine, 4-fluoro-D-phenylalanine, 4-fluoro-L-phenylalanine, 4-iodo-D-phenylalanine, 4-iodo-L-phenylalanine, homophenylalanine, thyroxine, 3,3-diphenylalanine, thyrone, ethyl-tyrosine, and methyl-tyrosine.

Amino acid analogs include analogs of proline. Examples of amino acid analogs of proline include, but are not limited to, 3,4-dehydro-proline, 4-fluoro-proline, cis-4-hydroxy-proline, thiazolidine-2-carboxylic acid, and trans-4-fluoroproline.

Amino acid analogs include analogs of serine and threonine. Examples of amino acid analogs of serine and threonine include, but are not limited to, 3-amino-2-hydroxy-5-methylhexanoic acid, 2-amino-3-hydroxy-4-methylpentanoic acid, 2-amino-3-ethoxybutanoic acid, 2-amino-3-methoxybutanoic acid, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-amino-3-benzyloxypionic acid, 2-amino-3-benzyloxypionic acid, 2-amino-3-ethoxypropionic acid, 4-amino-3-hydroxybutanoic acid, and α -methylserine.

25

Amino acid analogs include analogs of tryptophan. Examples of amino acid analogs of tryptophan include, but are not limited to, the following: α -methyl-tryptophan; β -(3-benzothienyl)-D-alanine; β -(3-benzothienyl)-L-alanine; 1-methyl-tryptophan; 4-methyl-tryptophan; 5-benzoyloxy-tryptophan; 5-bromo-tryptophan; 5-chloro-tryptophan; 5-fluoro-tryptophan; 5-hydroxy-tryptophan; 5-hydroxy-L-tryptophan; 5-methoxy-tryptophan; 5-methoxy-L-tryptophan; 5-methyl-tryptophan; 6-bromo-tryptophan; 6-chloro-D-tryptophan; 6-chloro-tryptophan; 6-fluoro-tryptophan; 6-methyl-tryptophan; 7-benzoyloxy-tryptophan; 7-bromo-tryptophan; 7-methyl-tryptophan; D-1,2,3,4-tetrahydronorharman-3-carboxylic acid; 6-methoxy-1,2,3,4-tetrahydronorharman-1-carboxylic acid; 7-azatryptophan; L-1,2,3,4-tetrahydronorharman-3-carboxylic acid; 5-methoxy-2-methyl-tryptophan; and 6-chloro-L-tryptophan.

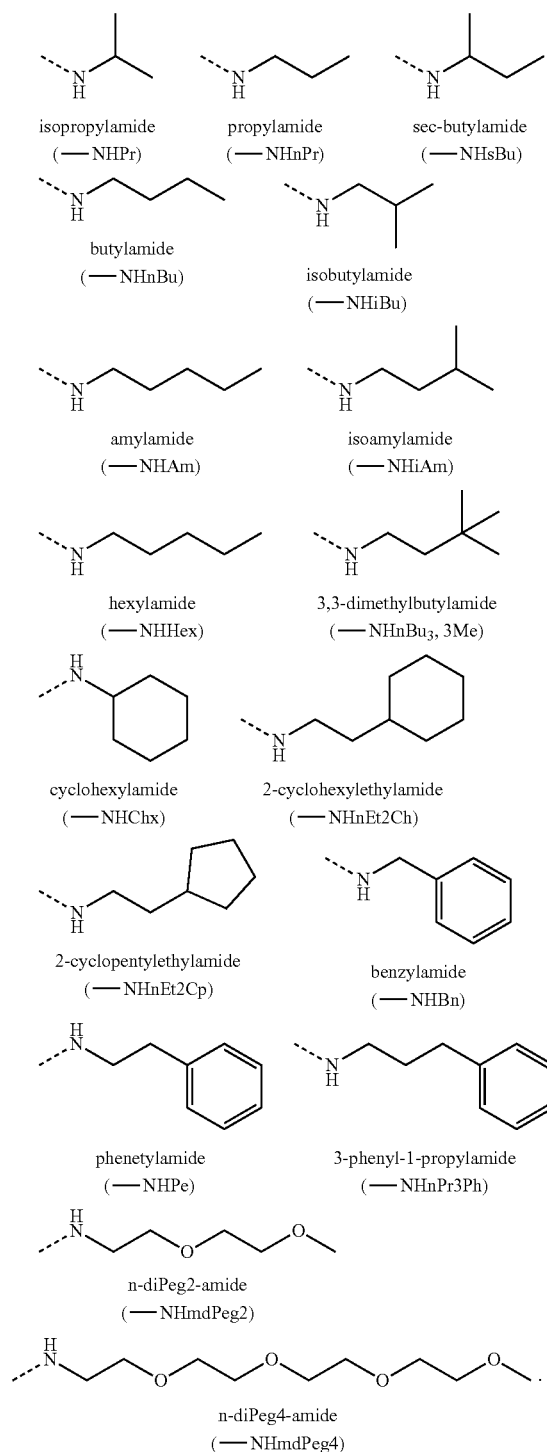
In some embodiments, amino acid analogs are racemic. In some embodiments, the D isomer of the amino acid analog is used. In some embodiments, the L isomer of the amino acid analog is used. In other embodiments, the amino acid analog comprises chiral centers that are in the R or S configuration. In still other embodiments, the amino group(s) of a β -amino acid analog is substituted with a protecting group, e.g., tert-butyloxycarbonyl (BOC group), 9-fluorenylmethyloxycarbonyl (Fmoc), tosyl, and the like. In yet other embodiments, the carboxylic acid functional group of a β -amino acid analog is protected, e.g., as its ester derivative. In some embodiments the salt of the amino acid analog is used.

A “non-essential” amino acid residue is a residue that can be altered from the wild-type sequence of a polypeptide without abolishing or substantially altering its essential biological or biochemical activity (e.g., receptor binding or activation). An “essential” amino acid residue is a residue that, when altered from the wild-type sequence of the polypeptide, results in abolishing or substantially abolishing the polypeptide’s essential biological or biochemical activity.

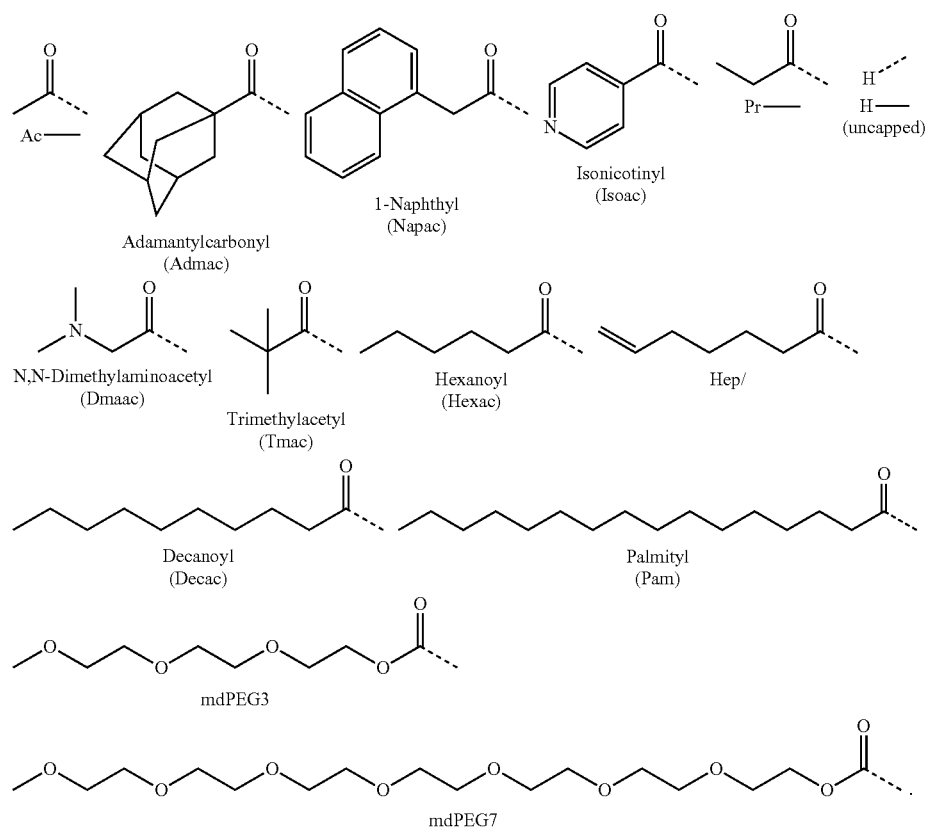
A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., K, R, H), acidic side chains (e.g., D, E), uncharged polar side chains (e.g., G, N, Q, S, T, Y, C), nonpolar side chains (e.g., A, V, L, I, P, F, M, W), beta-branched side chains (e.g., T, V, I) and aromatic side chains (e.g., Y, F, W, H). Thus, a predicted nonessential amino acid residue in a polypeptide, for example, is replaced with another amino acid residue from the same side chain family. Other examples of acceptable substitutions are substitutions based on isosteric considerations (e.g. norleucine for methionine) or other properties (e.g. 2-thienylalanine for phenylalanine, or 6-Cl-tryptophan for tryptophan).

The term “capping group” refers to the chemical moiety occurring at either the carboxy or amino terminus of the polypeptide chain of the subject peptidomimetic macrocycle. The capping group of a carboxy terminus includes an unmodified carboxylic acid (ie —COOH) or a carboxylic acid with a substituent. For example, the carboxy terminus can be substituted with an amino group to yield a carboxamide at the C-terminus. Various substituents include but are not limited to primary and secondary amines, including pegylated secondary amines. Representative secondary amine capping groups for the C-terminus include:

26



The capping group of an amino terminus includes an unmodified amine (ie —NH_2) or an amine with a substituent. For example, the amino terminus can be substituted with an acyl group to yield a carboxamide at the N-terminus. Various substituents include but are not limited to substituted acyl groups, including $\text{C}_1\text{—C}_6$ carbonyls, $\text{C}_7\text{—C}_{30}$ carbonyls, and pegylated carbamates. Representative capping groups for the N-terminus include, but are not limited to, 4-FBzl (4-fluoro-benzyl) and the following:



The term “member” as used herein in conjunction with macrocycles or macrocycle-forming linkers refers to the atoms that form or can form the macrocycle, and excludes substituent or side chain atoms. By analogy, cyclodecane, 1,2-difluoro-decane and 1,3-dimethyl cyclodecane are all considered ten-membered macrocycles as the hydrogen or fluoro substituents or methyl side chains do not participate in forming the macrocycle.

The symbol “//” when used as part of a molecular structure refers to a single bond or a trans or cis double bond.

The term “amino acid side chain” refers to a moiety attached to the α -carbon (or another backbone atom) in an amino acid. For example, the amino acid side chain for alanine is methyl, the amino acid side chain for phenylalanine is phenylmethyl, the amino acid side chain for cysteine is thiomethyl, the amino acid side chain for aspartate is carboxymethyl, the amino acid side chain for tyrosine is 4-hydroxyphenylmethyl, etc. Other non-naturally occurring amino acid side chains are also included, for example, those that occur in nature (e.g., an amino acid metabolite) or those that are made synthetically (e.g., an α,α di-substituted amino acid).

The term “ α,α di-substituted amino” acid refers to a molecule or moiety containing both an amino group and a carboxyl group bound to a carbon (the α -carbon) that is attached to two natural or non-natural amino acid side chains.

The term “polypeptide” encompasses two or more naturally or non-naturally-occurring amino acids joined by a covalent bond (e.g., an amide bond). Polypeptides as described herein include full length proteins (e.g., fully

processed proteins) as well as shorter amino acid sequences (e.g., fragments of naturally-occurring proteins or synthetic polypeptide fragments).

The term “first C-terminal amino acid” refers to the amino acid which is closest to the C-terminus. The term “second C-terminal amino acid” refers to the amino acid attached at the N-terminus of the first C-terminal amino acid.

The term “macrocyclization reagent” or “macrocycle-forming reagent” as used herein refers to any reagent which can be used to prepare a peptidomimetic macrocycle by mediating the reaction between two reactive groups. Reactive groups can be, for example, an azide and alkyne, in which case macrocyclization reagents include, without limitation, Cu reagents such as reagents which provide a reactive Cu(I) species, such as CuBr, CuI or CuOTf, as well as Cu(II) salts such as Cu(CO₂CH₃)₂, CuSO₄, and CuCl₂ that can be converted in situ to an active Cu(I) reagent by the addition of a reducing agent such as ascorbic acid or sodium ascorbate. Macrocyclization reagents can additionally include, for example, Ru reagents known in the art such as Cp*RuCl(PPh₃)₂, [Cp*RuCl]₄ or other Ru reagents which can provide a reactive Ru(II) species. In other cases, the reactive groups are terminal olefins. In such embodiments, the macrocyclization reagents or macrocycle-forming reagents are metathesis catalysts including, but not limited to, stabilized, late transition metal carbene complex catalysts such as Group VIII transition metal carbene catalysts. For example, such catalysts are Ru and Os metal centers having a +2 oxidation state, an electron count of 16 and pentacoordinated. In other examples, catalysts have W or Mo centers. Various catalysts are disclosed in Grubbs et al., “Ring Closing Metathesis and Related Processes in Organic Synthesis” Acc. Chem. Res.

1995, 28, 446-452, U.S. Pat. Nos. 5,811,515; 7,932,397; U.S. Application No. 2011/0065915; U.S. Application No. 2011/0245477; Yu et al., "Synthesis of Macrocyclic Natural Products by Catalyst-Controlled Stereoselective Ring-Closing Metathesis," *Nature* 2011, 479, 88; and Peryshkov et al., "Z-Selective Olefin Metathesis Reactions Promoted by Tungsten Oxo Alkylidene Complexes," *J. Am. Chem. Soc.* 2011, 133, 20754. In yet other cases, the reactive groups are thiol groups. In such embodiments, the macrocyclization reagent is, for example, a linker functionalized with two thiol-reactive groups such as halogen groups.

The term "halo" or "halogen" refers to fluorine, chlorine, bromine or iodine or a radical thereof.

The term "alkyl" refers to a hydrocarbon chain that is a straight chain or branched chain, containing the indicated number of carbon atoms. For example, C₁-C₁₀ indicates that the group has from 1 to 10 (inclusive) carbon atoms in it. In the absence of any numerical designation, "alkyl" is a chain (straight or branched) having 1 to 20 (inclusive) carbon atoms in it.

The term "alkylene" refers to a divalent alkyl (i.e., —R—).

The term "alkenyl" refers to a hydrocarbon chain that is a straight chain or branched chain having one or more carbon-carbon double bonds. The alkenyl moiety contains the indicated number of carbon atoms. For example, C₂-C₁₀ indicates that the group has from 2 to 10 (inclusive) carbon atoms in it. The term "lower alkenyl" refers to a C₂-C₆ alkenyl chain. In the absence of any numerical designation, "alkenyl" is a chain (straight or branched) having 2 to 20 (inclusive) carbon atoms in it.

The term "alkynyl" refers to a hydrocarbon chain that is a straight chain or branched chain having one or more carbon-carbon triple bonds. The alkynyl moiety contains the indicated number of carbon atoms. For example, C₂-C₁₀ indicates that the group has from 2 to 10 (inclusive) carbon atoms in it. The term "lower alkynyl" refers to a C₂-C₆ alkynyl chain. In the absence of any numerical designation, "alkynyl" is a chain (straight or branched) having 2 to 20 (inclusive) carbon atoms in it.

The term "aryl" refers to a 6-carbon monocyclic or 10-carbon bicyclic aromatic ring system wherein 0, 1, 2, 3, or 4 atoms of each ring are substituted by a substituent. Examples of aryl groups include phenyl, naphthyl and the like. The term "arylalkoxy" refers to an alkoxy substituted with aryl.

"Arylalkyl" refers to an aryl group, as defined above, wherein one of the aryl group's hydrogen atoms has been replaced with a C₁-C₅ alkyl group, as defined above. Representative examples of an arylalkyl group include, but are not limited to, 2-methylphenyl, 3-methylphenyl, 4-methylphenyl, 2-ethylphenyl, 3-ethylphenyl, 4-ethylphenyl, 2-propylphenyl, 3-propylphenyl, 4-propylphenyl, 2-butylphenyl, 3-butylphenyl, 4-butylphenyl, 2-pentylphenyl, 3-pentylphenyl, 4-pentylphenyl, 2-isopropylphenyl, 3-isopropylphenyl, 4-isopropylphenyl, 2-isobutylphenyl, 3-isobutylphenyl, 4-isobutylphenyl, 2-sec-butylphenyl, 3-sec-butylphenyl, 4-sec-butylphenyl, 2-t-butylphenyl, 3-t-butylphenyl and 4-t-butylphenyl.

"Arylamido" refers to an aryl group, as defined above, wherein one of the aryl group's hydrogen atoms has been replaced with one or more —C(O)NH₂ groups. Representative examples of an arylamido group include 2-C(O)NH₂-phenyl, 3-C(O)NH₂-phenyl, 4-C(O)NH₂-phenyl, 2-C(O)NH₂-pyridyl, 3-C(O)NH₂-pyridyl, and 4-C(O)NH₂-pyridyl.

"Alkylheterocycle" refers to a C₁-C₅ alkyl group's

hydrogen atoms has been replaced with a heterocycle. Representative examples of an alkylheterocycle group include, but are not limited to, —CH₂CH₂-morpholine, —CH₂CH₂-piperidine, —CH₂CH₂CH₂-morpholine, and —CH₂CH₂CH₂-imidazole.

"Alkylamido" refers to a C₁-C₅ alkyl group, as defined above, wherein one of the C₁-C₅ alkyl group's hydrogen atoms has been replaced with a —C(O)NH₂ group. Representative examples of an alkylamido group include, but are not limited to, —CH₂—C(O)NH₂, —CH₂CH₂—C(O)NH₂, —CH₂CH₂CH₂—C(O)NH₂, —CH₂CH₂CH₂CH₂—C(O)NH₂, —CH₂CH₂CH₂CH₂CH₂—C(O)NH₂, —CH₂CH(C(O)NH₂)CH₃, —CH₂CH(C(O)NH₂)CH₂CH₃, —CH(C(O)NH₂)CH₂CH₃, —C(CH₃)₂CH₂—C(O)NH₂, —CH₂—CH₂—NH—C(O)—CH₃, —CH₂—CH₂—NH—C(O)—CH₂—CH₃, and —CH₂—CH₂—NH—C(O)—CH=CH₂.

"Alkanol" refers to a C₁-C₅ alkyl group, as defined above, wherein one of the C₁-C₅ alkyl group's hydrogen atoms has been replaced with a hydroxyl group. Representative examples of an alkanol group include, but are not limited to, —CH₂OH, —CH₂CH₂OH, —CH₂CH₂CH₂OH, —CH₂CH₂CH₂CH₂OH, —CH₂CH₂CH₂CH₂CH₂OH, —CH₂CH(OH)CH₃, —CH₂CH(OH)CH₂CH₃, —CH(OH)CH₃ and —C(CH₃)₂CH₂OH.

"Alkylcarboxy" refers to a C₁-C₅ alkyl group, as defined above, wherein one of the C₁-C₅ alkyl group's hydrogen atoms has been replaced with a —COOH group. Representative examples of an alkylcarboxy group include, but are not limited to, —CH₂COOH, —CH₂CH₂COOH, —CH₂CH₂CH₂COOH, —CH₂CH₂CH₂CH₂COOH, —CH₂CH(COOH)CH₃, —CH₂CH₂CH₂CH₂CH₂COOH, —CH₂CH(COOH)CH₂CH₃, —CH(COOH)CH₂CH₃ and —C(CH₃)₂CH₂COOH.

The term "cycloalkyl" as employed herein includes saturated and partially unsaturated cyclic hydrocarbon groups having 3 to 12 carbons, preferably 3 to 8 carbons, and more preferably 3 to 6 carbons, wherein the cycloalkyl group additionally is optionally substituted. Some cycloalkyl groups include, without limitation, cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclohexyl, cyclohexenyl, cycloheptyl, and cyclooctyl.

The term "heteroaryl" refers to an aromatic 5-8 membered monocyclic, 8-12 membered bicyclic, or 11-14 membered tricyclic ring system having 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, said heteroatoms selected from O, N, or S (e.g., carbon atoms and 1-3, 1-6, or 1-9 heteroatoms of O, N, or S if monocyclic, bicyclic, or tricyclic, respectively), wherein 0, 1, 2, 3, or 4 atoms of each ring are substituted by a substituent. Examples of heteroaryl groups include pyridyl, furyl or furanyl, imidazolyl, benzimidazolyl, pyrimidinyl, thiophenyl or thienyl, quinolinyl, indolyl, thiazolyl, and the like.

The term "heteroarylalkyl" or the term "heteroaralkyl" refers to an alkyl substituted with a heteroaryl. The term "heteroarylalkoxy" refers to an alkoxy substituted with heteroaryl.

The term "heteroarylalkyl" or the term "heteroaralkyl" refers to an alkyl substituted with a heteroaryl. The term "heteroarylalkoxy" refers to an alkoxy substituted with heteroaryl.

The term "heterocyclyl" refers to a nonaromatic 5-8 membered monocyclic, 8-12 membered bicyclic, or 11-14 membered tricyclic ring system having 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, said heteroatoms selected from O, N, or S (e.g., carbon atoms and 1-3, 1-6, or 1-9 heteroatoms of O, N, or

31

S if monocyclic, bicyclic, or tricyclic, respectively), wherein 0, 1, 2 or 3 atoms of each ring are substituted by a substituent. Examples of heterocyclyl groups include piperazinyl, pyrrolidinyl, dioxanyl, morpholinyl, tetrahydrofuranyl, and the like.

The term “substituent” refers to a group replacing a second atom or group such as a hydrogen atom on any molecule, compound or moiety. Suitable substituents include, without limitation, halo, hydroxy, mercapto, oxo, nitro, haloalkyl, alkyl, alkaryl, aryl, aralkyl, alkoxy, thioalkoxy, aryloxy, amino, alkoxy carbonyl, amido, carboxy, alkanesulfonyl, alkyl carbonyl, and cyano groups.

In some embodiments, the compounds disclosed herein contain one or more asymmetric centers and thus occur as racemates and racemic mixtures, single enantiomers, individual diastereomers and diastereomeric mixtures. All such isomeric forms of these compounds are included unless expressly provided otherwise. In some embodiments, the compounds disclosed herein are also represented in multiple tautomeric forms, in such instances, the compounds include all tautomeric forms of the compounds described herein (e.g., if alkylation of a ring system results in alkylation at multiple sites, the invention includes all such reaction products). All such isomeric forms of such compounds are included unless expressly provided otherwise. All crystal forms of the compounds described herein are included unless expressly provided otherwise.

As used herein, the terms “increase” and “decrease” mean, respectively, to cause a statistically significantly (i.e., $p < 0.1$) increase or decrease of at least 5%.

As used herein, the recitation of a numerical range for a variable is intended to convey that the variable is equal to any of the values within that range. Thus, for a variable which is inherently discrete, the variable is equal to any integer value within the numerical range, including the end-points of the range. Similarly, for a variable which is inherently continuous, the variable is equal to any real value within the numerical range, including the end-points of the range. As an example, and without limitation, a variable which is described as having values between 0 and 2 takes the values 0, 1 or 2 if the variable is inherently discrete, and takes the values 0.0, 0.1, 0.01, 0.001, or any other real values ≥ 0 and ≤ 2 if the variable is inherently continuous.

As used herein, unless specifically indicated otherwise, the word “or” is used in the inclusive sense of “and/or” and not the exclusive sense of “either/or.”

The term “on average” represents the mean value derived from performing at least three independent replicates for each data point.

The term “biological activity” encompasses structural and functional properties of a macrocycle. Biological activity is, for example, structural stability, alpha-helicity, affinity for a target, resistance to proteolytic degradation, cell penetrability, intracellular stability, in vivo stability, or any combination thereof.

The term “binding affinity” refers to the strength of a binding interaction, for example between a peptidomimetic macrocycle and a target. Binding affinity can be expressed, for example, as an equilibrium dissociation constant (“ K_D ”), which is expressed in units which are a measure of concentration (e.g. M, mM, μ M, nM etc). Numerically, binding affinity and K_D values vary inversely, such that a lower binding affinity corresponds to a higher K_D value, while a higher binding affinity corresponds to a lower K_D value. Where high binding affinity is desirable, “improved” binding affinity refers to higher binding affinity and therefore lower K_D values.

32

The term “in vitro efficacy” refers to the extent to which a test compound, such as a peptidomimetic macrocycle, produces a beneficial result in an in vitro test system or assay. In vitro efficacy can be measured, for example, as an “ IC_{50} ” or “ EC_{50} ” value, which represents the concentration of the test compound which produces 50% of the maximal effect in the test system.

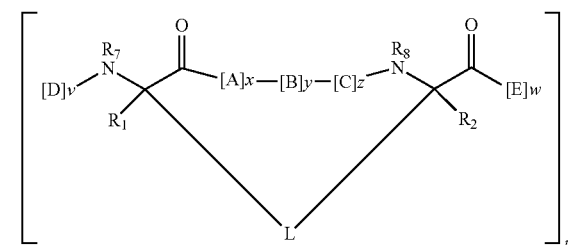
The term “ratio of in vitro efficacies” or “in vitro efficacy ratio” refers to the ratio of IC_{50} or EC_{50} values from a first assay (the numerator) versus a second assay (the denominator). Consequently, an improved in vitro efficacy ratio for Assay 1 versus Assay 2 refers to a lower value for the ratio expressed as $IC_{50}(\text{Assay 1})/IC_{50}(\text{Assay 2})$ or alternatively as $EC_{50}(\text{Assay 1})/EC_{50}(\text{Assay 2})$. This concept can also be characterized as “improved selectivity” in Assay 1 versus Assay 2, which can be due either to a decrease in the IC_{50} or EC_{50} value for Target 1 or an increase in the value for the IC_{50} or EC_{50} value for Target 2.

The details of one or more particular embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

Peptidomimetic Macrocycles

In some embodiments, a peptidomimetic macrocycle has the Formula (I):

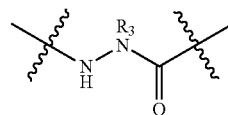
Formula I



wherein:

each A, C, D, and E is independently an amino acid (including natural or non-natural amino acids, and amino acid analogs) and the terminal D and E independently optionally include a capping group;

B is an amino acid (including natural or non-natural amino acids, and amino acid analogs),



[—NH—L₃—CO—], [—NH—L₃—SO₂—], or [—NH—L₃—];

R₁ and R₂ are independently —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo-;

R₃ is hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R₅;

L is a macrocycle-forming linker of the formula —L₁—L₂—;

L₁ and L₂ are independently alkylene, alkenylene, alkenylene, heteroalkylene, cycloalkylene, heterocycloalkylene, cycloarylene, heterocycloarylene, or [—R₄—K—R₄—]_m, each being optionally substituted with R₅;

33

each R_4 is alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene;

each K is O, S, SO, SO₂, CO, CO₂, or CONR₃;

each R_5 is independently halogen, alkyl, —OR₆, —N(R₆)₂, —SR₆, —SOR₆, —SO₂R₆, —CO₂R₆, a fluorescent moiety, a radioisotope or a therapeutic agent;

each R_6 is independently —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heterocycloalkyl, a fluorescent moiety, a radioisotope or a therapeutic agent;

R_7 is —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R_5 , or part of a cyclic structure with a D residue;

R_8 is —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R_5 , or part of a cyclic structure with an E residue;

v and w are independently integers from 1-1000, for example 1-500, 1-200, 1-100, 1-50, 1-30, 1-20, or 1-10;

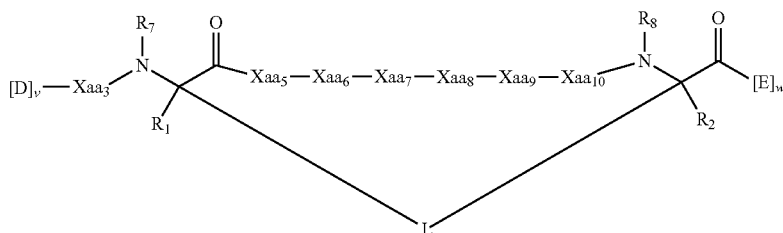
u is an integer from 1-10, for example 1-5, 1-3 or 1-2;

x , y and z are independently integers from 0-10, for example the sum of $x+y+z$ is 2, 3, or 6; and

n is an integer from 1-5.

In some embodiments, v and w are integers between 1-30. In some embodiments, w is an integer from 3-1000, for example 3-500, 3-200, 3-100, 3-50, 3-30, 3-20, or 3-10. In some embodiments, the sum of $x+y+z$ is 3 or 6. In some embodiments, the sum of $x+y+z$ is 3. In other embodiments, the sum of $x+y+z$ is 6.

In some embodiments, peptidomimetic macrocycles are also provided of the formula:



wherein:

each of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ is individually an amino acid, wherein at least three of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the corresponding position of the sequence Phe₃-X₄-His₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀-X₁₁-Ser₁₂ (SEQ ID NO: 8), where each X is an amino acid;

each D and E is independently an amino acid;

R_1 and R_2 are independently —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo-; or at least one of R_1 and R_2 forms a macrocycle-forming linker L' connected to the alpha position of one of said D or E amino acids;

each L or L' is independently a macrocycle-forming linker of the formula —L₁-L₂-;

L_1 and L_2 are independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, cycloarylene, heterocycloarylene, or [—R₄—K—R₄—]_n, each being optionally substituted with R_5 ;

R_3 is hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R_5 ;

34

each R_4 is alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene;

each K is O, S, SO, SO₂, CO, CO₂, or CONR₃;

each R_5 is independently halogen, alkyl, —OR₆, —N(R₆)₂, —SR₆, —SOR₆, —SO₂R₆, —CO₂R₆, a fluorescent moiety, a radioisotope or a therapeutic agent;

each R_6 is independently —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heterocycloalkyl, a fluorescent moiety, a radioisotope or a therapeutic agent;

R_7 is —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R_5 , or part of a cyclic structure with a D residue;

R_8 is —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R_5 , or part of a cyclic structure with an E residue;

v is an integer from 1-1000, for example 1-500, 1-200, 1-100, 1-50, 1-30, 1-20 or 1-10;

w is an integer from 3-1000, for example 3-500, 3-200, 3-100, 3-50, 3-30, 3-20, or 3-10; and

n is an integer from 1-5.

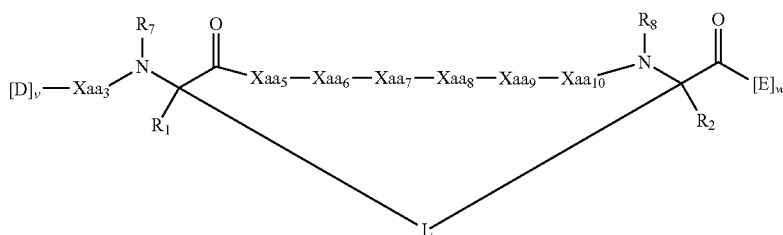
In some embodiments, v and w are integers between 1-30.

In some embodiments, w is an integer from 3-1000, for example 3-500, 3-200, 3-100, 3-50, 3-30, 3-20, or 3-10. In some embodiments, the sum of $x+y+z$ is 3 or 6. In some embodiments, the sum of $x+y+z$ is 3. In other embodiments, the sum of $x+y+z$ is 6.

In some embodiments of any of the Formulas described herein, at least three of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the

corresponding position of the sequence Phe₃-X₄-His₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀-X₁₁-Ser₁₂ (SEQ ID NO: 8). In other embodiments, at least four of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the corresponding position of the sequence Phe₃-X₄-His₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀-X₁₁-Ser₁₂ (SEQ ID NO: 8). In other embodiments, at least five of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the corresponding position of the sequence Phe₃-X₄-His₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀-X₁₁-Ser₁₂ (SEQ ID NO: 8). In other embodiments, at least six of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the corresponding position of the sequence Phe₃-X₄-His₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀-X₁₁-Ser₁₂ (SEQ ID NO: 8). In other embodiments, at least seven of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the corresponding position of the sequence Phe₃-X₄-His₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀-X₁₁-Ser₁₂ (SEQ ID NO: 8).

In some embodiments, a peptidomimetic macrocycle has the Formula:



wherein:

each of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ is individually an amino acid, wherein at least three of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the corresponding position of the sequence Phe₃-X₄-Glu₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀/Cba₁₀-X₁₁-Ala₁₂ (SEQ ID NO: 9), where each X is an amino acid;

each D is independently an amino acid;

each E is independently an amino acid, for example an amino acid selected from Ala (alanine), D-Ala (D-alanine), Aib (α -aminoisobutyric acid), Sar (N-methyl glycine), and Ser (serine);

R₁ and R₂ are independently —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo-; or at least one of R₁ and R₂ forms a macrocycle-forming linker L' connected to the alpha position of one of said D or E amino acids;

each L or L' is independently a macrocycle-forming linker of the formula —L₁—L₂—;

L₁ and L₂ are independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, cycloarylene, heterocycloarylene, or [—R₄—K—R₄—]_n, each being optionally substituted with R₅;

R₃ is hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R₅;

each R₄ is alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylenylene, or heteroarylenylene;

each K is O, S, SO, SO₂, CO, CO₂, or CONR₃;

each R₅ is independently halogen, alkyl, —OR₆, —N(R₆)₂, —SR₆, —SOR₆, —SO₂R₆, —CO₂R₆, a fluorescent moiety, a radioisotope or a therapeutic agent;

each R₆ is independently —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heterocycloalkyl, a fluorescent moiety, a radioisotope or a therapeutic agent;

R₇ is —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R₅, or part of a cyclic structure with a D residue;

R₈ is —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R₅, or part of a cyclic structure with an E residue;

v is an integer from 1-1000, for example 1-500, 1-200, 1-100, 1-50, 1-30, 1-20, or 1-10;

w is an integer from 3-1000, for example 3-500, 3-200, 3-100, 3-50, 3-30, 3-20, or 3-10; and

n is an integer from 1-5.

In some embodiments of the above Formula, at least three of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the corresponding

position of the sequence Phe₃-X₄-Glu₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀/Cba₁₀-X₁₁-Ala₁₂ (SEQ ID NO: 9). In other embodiments of the above Formula, at least four of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the corresponding position of the sequence Phe₃-X₄-Glu₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀/Cba₁₀-X₁₁-Ala₁₂ (SEQ ID NO: 9). In other embodiments of the above Formula, at least five of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the corresponding position of the sequence Phe₃-X₄-Glu₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀/Cba₁₀-X₁₁-Ala₁₂ (SEQ ID NO: 9). In other embodiments of the above Formula, at least six of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the corresponding position of the sequence Phe₃-X₄-Glu₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀/Cba₁₀-X₁₁-Ala₁₂ (SEQ ID NO: 9). In other embodiments of the above Formula, at least seven of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acid as the amino acid at the corresponding position of the sequence Phe₃-X₄-Glu₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀/Cba₁₀-X₁₁-Ala₁₂ (SEQ ID NO: 9).

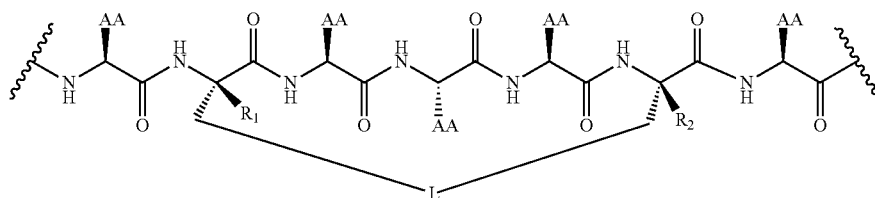
In some embodiments, w is an integer from 3-10, for example 3-6, 3-8, 6-8, or 6-10. In some embodiments, w is 3. In other embodiments, w is 6. In some embodiments, v is an integer from 1-10, for example 2-5. In some embodiments, v is 2.

In an embodiment of any of the Formulas described herein, L₁ and L₂, either alone or in combination, do not form a triazole or a thioether.

In one example, at least one of R₁ and R₂ is alkyl, unsubstituted or substituted with halo-. In another example, both R₁ and R₂ are independently alkyl, unsubstituted or substituted with halo-. In some embodiments, at least one of R₁ and R₂ is methyl. In other embodiments, R₁ and R₂ are methyl.

In some embodiments, x+y+z is at least 3. In other embodiments, x+y+z is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. In some embodiments, the sum of x+y+z is 3 or 6. In some embodiments, the sum of x+y+z is 3. In other embodiments, the sum of x+y+z is 6. Each occurrence of A, B, C, D or E in a macrocycle or macrocycle precursor is independently selected. For example, a sequence represented by the formula [A]_x, when x is 3, encompasses embodiments where the amino acids are not identical, e.g. Gln-Asp-Ala as well as embodiments where the amino acids are identical, e.g. Gln-Gln-Gln. This applies for any value of x, y, or z in the indicated ranges. Similarly, when u is greater than 1, each compound can encompass peptidomimetic macrocycles which are the same or different. For example, a compound can comprise peptidomimetic macrocycles comprising different linker lengths or chemical compositions.

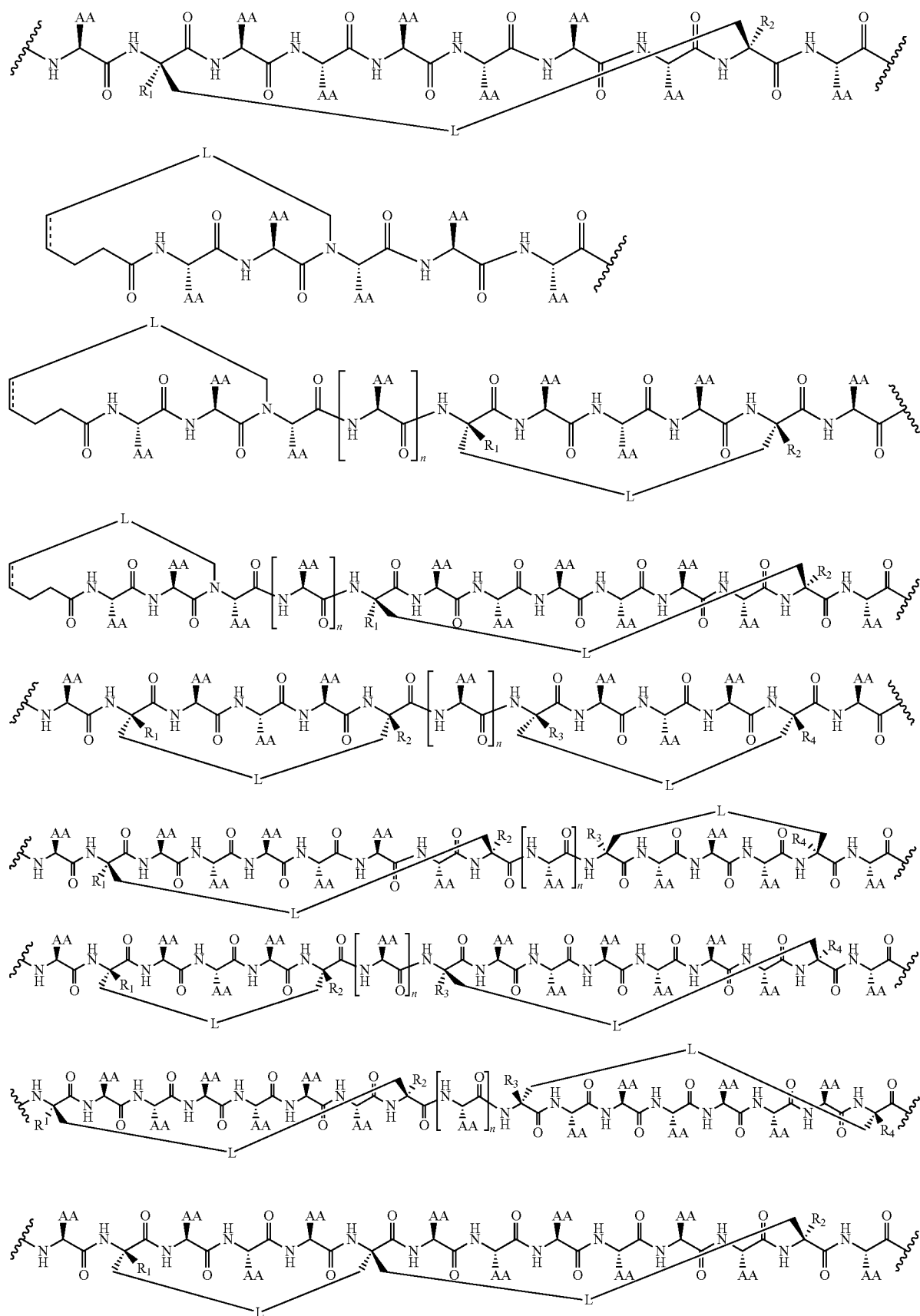
In some embodiments, the peptidomimetic macrocycle comprises a secondary structure which is an α -helix and R₈ is —H, allowing intrahelical hydrogen bonding. In some embodiments, at least one of A, B, C, D or E is an α,α -disubstituted amino acid. In one example, B is an

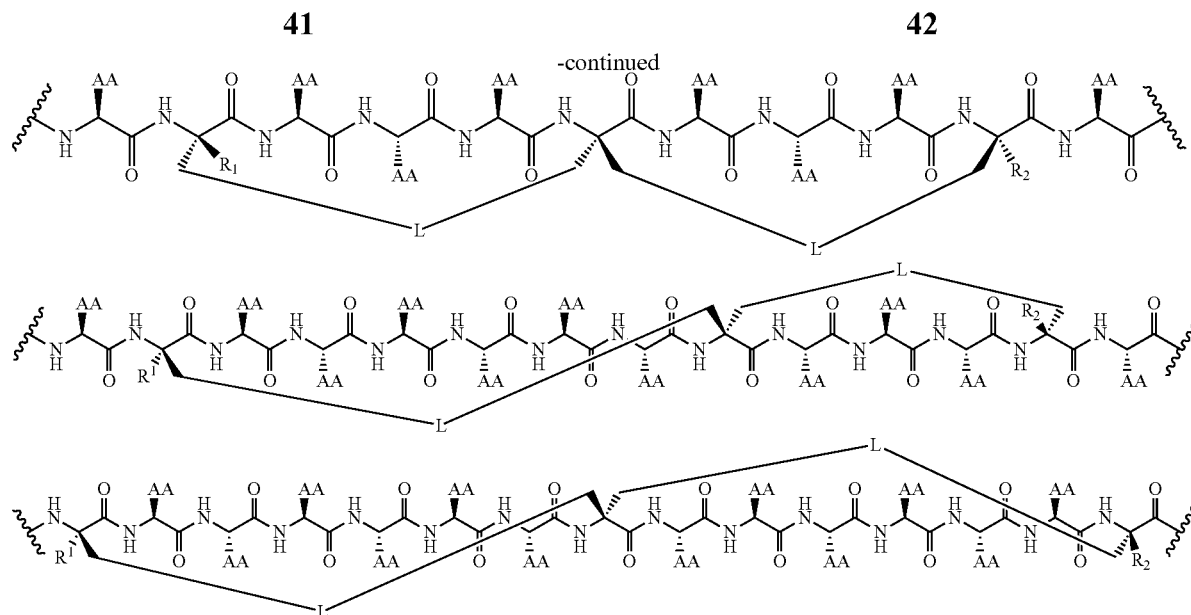


39

40

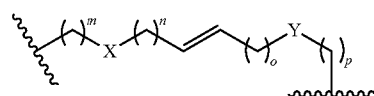
-continued



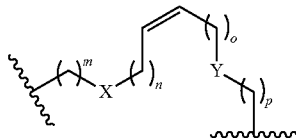


wherein "AA" represents any natural or non-natural amino acid side chain and "AA" is [D]_w, [E]_w as defined above, and n is an integer between 0 and 20, 50, 100, 200, 300, 400 or 500. In some embodiments, n is 0. In other embodiments, n is less than 50.

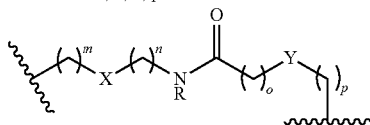
Exemplary embodiments of the macrocycle-forming linker L are shown below.



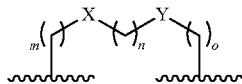
where X, Y = —CH₂—, O, S, or NH
m, n, o, p = 0-10



where X, Y = —CH₂—, O, S, or NH
m, n, o, p = 0-10



where X, Y = —CH₂—, O, S, or NH
m, n, o, p = 0-10
R = H, alkyl, other substituent



where X, Y = —CH₂—, O, S, or NH
m, n, o = 0-10

In other embodiments, D and/or E in the compound of Formula I are further modified in order to facilitate cellular uptake. In some embodiments, lipidating or PEGylating a

peptidomimetic macrocycle facilitates cellular uptake, increases bioavailability, increases blood circulation, alters pharmacokinetics, decreases immunogenicity and/or decreases the needed frequency of administration.

In other embodiments, at least one of [D] and [E] in the compound of Formula I represents a moiety comprising an additional macrocycle-forming linker such that the peptidomimetic macrocycle comprises at least two macrocycle-forming linkers. In a specific embodiment, a peptidomimetic macrocycle comprises two macrocycle-forming linkers. In an embodiment, u is 2.

In some embodiments, any of the macrocycle-forming linkers described herein can be used in any combination with any of the sequences shown in Table 1, Table 1a, Table 1b, or Table 1c and also with any of the R-substituents indicated herein.

In some embodiments, the peptidomimetic macrocycle comprises at least one α -helix motif. For example, A, B and/or C in the compound of Formula I include one or more α -helices. As a general matter, α -helices include between 3 and 4 amino acid residues per turn. In some embodiments, the α -helix of the peptidomimetic macrocycle includes 1 to 5 turns and, therefore, 3 to 20 amino acid residues. In specific embodiments, the α -helix includes 1 turn, 2 turns, 3 turns, 4 turns, or 5 turns. In some embodiments, the macrocycle-forming linker stabilizes an α -helix motif included within the peptidomimetic macrocycle. Thus, in some embodiments, the length of the macrocycle-forming linker L from a first C α to a second C α is selected to increase the stability of an α -helix. In some embodiments, the macrocycle-forming linker spans from 1 turn to 5 turns of the α -helix. In some embodiments, the macrocycle-forming linker spans approximately 1 turn, 2 turns, 3 turns, 4 turns, or 5 turns of the α -helix. In some embodiments, the length of the macrocycle-forming linker is approximately 5 Å to 9 Å per turn of the α -helix, or approximately 6 Å to 8 Å per turn of the α -helix. Where the macrocycle-forming linker spans approximately 1 turn of an α -helix, the length is equal to approximately 5 carbon-carbon bonds to 13 carbon-carbon bonds, approximately 7 carbon-carbon bonds to 11 carbon-carbon bonds, or approximately 9 carbon-carbon bonds. Where the macrocycle-forming linker spans approxi-

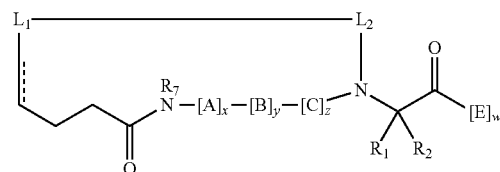
43

mately 2 turns of an α -helix, the length is equal to approximately 8 carbon-carbon bonds to 16 carbon-carbon bonds, approximately 10 carbon-carbon bonds to 14 carbon-carbon bonds, or approximately 12 carbon-carbon bonds. Where the macrocycle-forming linker spans approximately 3 turns of an α -helix, the length is equal to approximately 14 carbon-carbon bonds to 22 carbon-carbon bonds, approximately 16 carbon-carbon bonds to 20 carbon-carbon bonds, or approximately 18 carbon-carbon bonds. Where the macrocycle-forming linker spans approximately 4 turns of an α -helix, the length is equal to approximately 20 carbon-carbon bonds to 28 carbon-carbon bonds, approximately 22 carbon-carbon bonds to 26 carbon-carbon bonds, or approximately 24 carbon-carbon bonds. Where the macrocycle-forming linker spans approximately 5 turns of an α -helix, the length is equal to approximately 26 carbon-carbon bonds to 34 carbon-carbon bonds, approximately 28 carbon-carbon bonds to 32 carbon-carbon bonds, or approximately 30 carbon-carbon bonds. Where the macrocycle-forming linker spans approximately 1 turn of an α -helix, the linkage contains approximately 4 atoms to 12 atoms, approximately 6 atoms to 10 atoms, or approximately 8 atoms. Where the macrocycle-forming linker spans approximately 2 turns of the α -helix, the linkage contains approximately 7 atoms to 15 atoms, approximately 9 atoms to 13 atoms, or approximately 11 atoms. Where the macrocycle-forming linker spans approximately 3 turns of the α -helix, the linkage contains approximately 13 atoms to 21 atoms, approximately 15 atoms to 19 atoms, or approximately 17 atoms. Where the macrocycle-forming linker spans approximately 4 turns of the α -helix, the linkage contains approximately 19 atoms to 27 atoms, approximately 21 atoms to 25 atoms, or approximately 23 atoms. Where the macrocycle-forming linker spans approximately 5 turns of the α -helix, the linkage contains approximately 25 atoms to 33 atoms, approximately 27 atoms to 31 atoms, or approximately 29 atoms. Where the macrocycle-forming linker spans approximately 1 turn of the α -helix, the resulting macrocycle forms a ring containing approximately 17 members to 25 members, approximately 19 members to 23 members, or approximately 21 members. Where the macrocycle-forming linker spans approximately 2 turns of the α -helix, the resulting macrocycle forms a ring containing approximately 29 members to 37 members, approximately 31 members to 35 members, or approximately 33 members. Where the macrocycle-forming linker spans approximately 3 turns of the α -helix, the resulting macrocycle forms a ring containing approximately 44 members to 52 members, approximately 46 members to 50 members, or approximately 48 members. Where the macrocycle-forming linker spans approximately 4 turns of the α -helix, the resulting macrocycle forms a ring containing approximately 59 members to 67 members, approximately 61 members to 65 members, or approximately 63 members. Where the macrocycle-forming linker spans approximately 5 turns of the α -helix, the resulting macrocycle forms a ring containing approximately 74 members to 82 members, approximately 76 members to 80 members, or approximately 78 members.

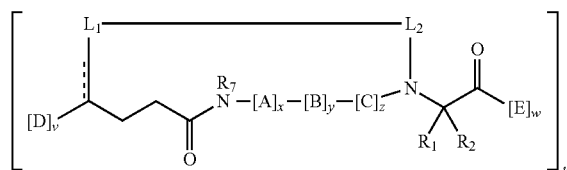
In other embodiments, provided are peptidomimetic macrocycles of Formula (IV) or (IVa):

44

Formula (IV)



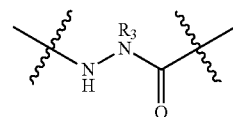
Formula (IVa)



wherein:

each A, C, D, and E is independently a natural or non-natural amino acid, and the terminal D and E independently optionally include a capping group;

B is a natural or non-natural amino acid, amino acid analog,



[—NH—L₃—CO—], [—NH—L₃—SO₂—], or [—NH—L₃—];

R₁ and R₂ are independently —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo-; or at least one of R₁ and R₂ forms a macrocycle-forming linker L' connected to the alpha position of one of said D or E amino acids;

R₃ is hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R₅;

L is a macrocycle-forming linker of the formula —L₁—L₂—;

L₁ and L₂ are independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, cycloarylene, heterocycloarylene, or [—R₄—K—R₄—]_n, each being optionally substituted with R₅;

each R₄ is alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene;

each K is O, S, SO, SO₂, CO, CO₂, or CONR₃;

each R₅ is independently halogen, alkyl, —OR₆, —N(R₆)₂, —SR₆, —SOR₆, —SO₂R₆, —CO₂R₆, a fluorescent moiety, a radioisotope or a therapeutic agent;

each R₆ is independently —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heterocycloalkyl, a fluorescent moiety, a radioisotope or a therapeutic agent;

R₇ is —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, cycloaryl, or heterocycloaryl, optionally substituted with R₅;

v and w are independently integers from 1-1000;

u is an integer from 1-10;

x, y and z are independently integers from 0-10; and

n is an integer from 1-5.

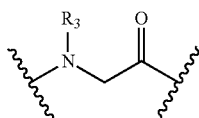
In one example, L₁ and L₂, either alone or in combination, do not form a triazole or a thioether.

45

In one example, at least one of R_1 and R_2 is alkyl, unsubstituted or substituted with halo-. In another example, both R_1 and R_2 are independently alkyl, unsubstituted or substituted with halo-. In some embodiments, at least one of R_1 and R_2 is methyl. In other embodiments, R_1 and R_2 are methyl.

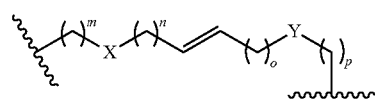
In some embodiments, $x+y+z$ is at least 1. In other embodiments, $x+y+z$ is at least 2. In other embodiments, $x+y+z$ is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. Each occurrence of A, B, C, D or E in a macrocycle or macrocycle precursor is independently selected. For example, a sequence represented by the formula $[A]_x$, when x is 3, encompasses embodiments where the amino acids are not identical, e.g. Gln-Asp-Ala as well as embodiments where the amino acids are identical, e.g. Gln-Gln-Gln. This applies for any value of x , y , or z in the indicated ranges.

In some embodiments, the peptidomimetic macrocycle comprises a secondary structure which is an α -helix and R_8 is $-H$, allowing intrahelical hydrogen bonding. In some embodiments, at least one of A, B, C, D or E is an α,α -disubstituted amino acid. In one example, B is an α,α -disubstituted amino acid. For instance, at least one of A, B, C, D or E is 2-aminoisobutyric acid. In other embodiments, at least one of A, B, C, D or E is

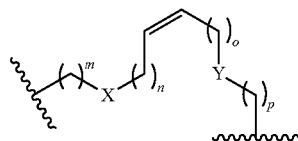


In other embodiments, the length of the macrocycle-forming linker L as measured from a first $C\alpha$ to a second $C\alpha$ is selected to stabilize a desired secondary peptide structure, such as an α -helix formed by residues of the peptidomimetic macrocycle including, but not necessarily limited to, those between the first $C\alpha$ to a second $C\alpha$.

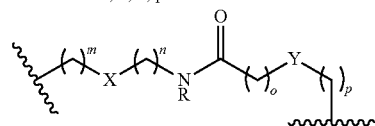
Exemplary embodiments of the macrocycle-forming linker $-L_1-L_2-$ are shown below.



where $X, Y = -CH_2-$, O, S, or NH
 $m, n, o, p = 0-10$



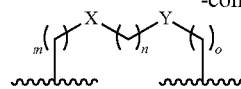
where $X, Y = -CH_2-$, O, S, or NH
 $m, n, o, p = 0-10$



where $X, Y = -CH_2-$, O, S, or NH
 $m, n, o, p = 0-10$
 $R = H$, alkyl, other substituent

46

-continued



where $X, Y = -CH_2-$, O, S, or NH
 $m, n, o = 0-10$

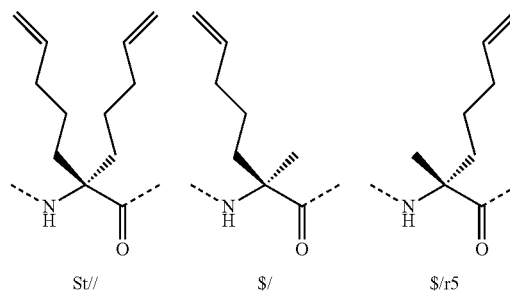
Unless otherwise stated, any compounds (including peptidomimetic macrocycles, macrocycle precursors, and other compositions) are also meant to encompass compounds which differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the described structures except for the replacement of a hydrogen by a deuterium or tritium, or the replacement of a carbon by ^{13}C - or ^{14}C -enriched carbon are within the scope of this invention.

In some embodiments, the compounds disclosed herein can contain unnatural proportions of atomic isotopes at one or more of atoms that constitute such compounds. For example, the compounds can be radiolabeled with radioactive isotopes, such as for example tritium (3H), iodine-125 (^{125}I) or carbon-14 (^{14}C). In other embodiments, one or more carbon atoms is replaced with a silicon atom. All isotopic variations of the compounds disclosed herein, whether radioactive or not, are contemplated herein.

Preparation of Peptidomimetic Macrocycles

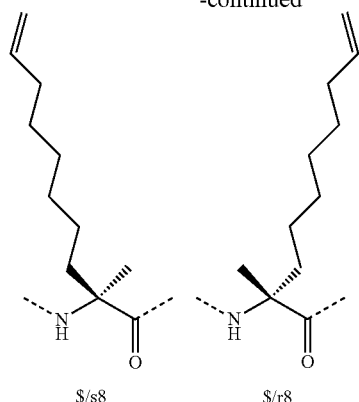
Peptidomimetic macrocycles can be prepared by any of a variety of methods known in the art. For example, any of the residues indicated by "S" or "Sr8" in Table 1, Table 1a, Table 1b, or Table 1c can be substituted with a residue capable of forming a crosslinker with a second residue in the same molecule or a precursor of such a residue.

Various methods to effect formation of peptidomimetic macrocycles are known in the art. For example, the preparation of peptidomimetic macrocycles of Formula I is described in Schafmeister et al., J. Am. Chem. Soc. 122: 5891-5892 (2000); Schafmeister & Verdine, J. Am. Chem. Soc. 122:5891 (2005); Walensky et al., Science 305:1466-1470 (2004); U.S. Pat. No. 7,192,713 and PCT application WO 2008/121767. The α,α -disubstituted amino acids and amino acid precursors disclosed in the cited references can be employed in synthesis of the peptidomimetic macrocycle precursor polypeptides. For example, the "S5-olefin amino acid" is (S)- α -(2'-pentenyl) alanine and the "R8 olefin amino acid" is (R)- α -(2'-octenyl) alanine. Following incorporation of such amino acids into precursor polypeptides, the terminal olefins are reacted with a metathesis catalyst, leading to the formation of the peptidomimetic macrocycle. In various embodiments, the following amino acids can be employed in the synthesis of the peptidomimetic macrocycle:



47

-continued



In other embodiments, the peptidomimetic macrocycles are of Formula IV or IVa. Methods for the preparation of such macrocycles are described, for example, in U.S. Pat. No. 7,202,332.

Additional methods of forming peptidomimetic macrocycles which are envisioned as suitable include those disclosed by Mustapa, M. Firouz Mohd et al., *J. Org. Chem.* (2003), 68, pp. 8193-8198; Yang, Bin et al. *Bioorg Med. Chem. Lett.* (2004), 14, pp. 1403-1406; U.S. Pat. Nos. 5,364,851; 5,446,128; 5,824,483; 6,713,280; and 7,202,332. In such embodiments, amino acid precursors are used containing an additional substituent R— at the alpha position. Such amino acids are incorporated into the macrocycle precursor at the desired positions, which can be at the positions where the crosslinker is substituted or, alternatively, elsewhere in the sequence of the macrocycle precursor. Cyclization of the precursor is then effected according to the indicated method.

Assays

The properties of peptidomimetic macrocycles are assayed, for example, by using the methods described below. In some embodiments, a peptidomimetic macrocycle has improved biological properties relative to a corresponding polypeptide lacking the substituents described herein.

Assay to Determine α -Helicity

In solution, the secondary structure of polypeptides with α -helical domains will reach a dynamic equilibrium between random coil structures and α -helical structures, often expressed as a “percent helicity”. Thus, for example, α -helical domains are predominantly random coils in solution, with α -helical content usually under 25%. Peptidomimetic macrocycles with optimized linkers, on the other hand, possess, for example, an α -helicity that is at least two-fold greater than that of a corresponding uncrosslinked polypeptide. In some embodiments, macrocycles will possess an α -helicity of greater than 50%. To assay the helicity of peptidomimetic macrocycles, the compounds are dissolved in an aqueous solution (e.g. 50 mM potassium phosphate solution at pH 7, or distilled H₂O, to concentrations of 25-50 μ M). Circular dichroism (CD) spectra are obtained on a spectropolarimeter (e.g., Jasco J-710) using standard measurement parameters (e.g. temperature, 20° C.; wavelength, 190-260 nm; step resolution, 0.5 nm; speed, 20 nm/sec; accumulations, 10; response, 1 sec; bandwidth, 1 nm; path length, 0.1 cm). The α -helical content of each peptide is calculated by dividing the mean residue ellipticity (e.g. $[\Phi]_{222}$ obs) by the reported value for a model helical decapeptide (Yang et al. (1986), *Methods Enzymol.* 130: 208)).

48

Assay to Determine Melting Temperature (T_m).

A peptidomimetic macrocycle comprising a secondary structure such as an α -helix exhibits, for example, a higher melting temperature than a corresponding uncrosslinked polypeptide. Typically peptidomimetic macrocycles exhibit T_m of >60° C. representing a highly stable structure in aqueous solutions. To assay the effect of macrocycle formation on melting temperature, peptidomimetic macrocycles or unmodified peptides are dissolved in distilled H₂O (e.g. at a final concentration of 50 μ M) and the T_m is determined by measuring the change in ellipticity over a temperature range (e.g. 4 to 95° C.) on a spectropolarimeter (e.g., Jasco J-710) using standard parameters (e.g. wavelength 222 nm; step resolution, 0.5 nm; speed, 20 nm/sec; accumulations, 10; response, 1 sec; bandwidth, 1 nm; temperature increase rate: 1° C./min; path length, 0.1 cm).

Protease Resistance Assay.

The amide bond of the peptide backbone is susceptible to hydrolysis by proteases, thereby rendering peptidic compounds vulnerable to rapid degradation in vivo. Peptide helix formation, however, typically buries the amide backbone and therefore can shield it from proteolytic cleavage. The peptidomimetic macrocycles can be subjected to in vitro trypsin proteolysis to assess for any change in degradation rate compared to a corresponding uncrosslinked polypeptide. For example, the peptidomimetic macrocycle and a corresponding uncrosslinked polypeptide are incubated with trypsin agarose and the reactions quenched at various time points by centrifugation and subsequent HPLC injection to quantitate the residual substrate by ultraviolet absorption at 280 nm. Briefly, the peptidomimetic macrocycle and peptidomimetic precursor (5 mcg) are incubated with trypsin agarose (Pierce) (S/E~125) for 0, 10, 20, 90, and 180 minutes. Reactions are quenched by tabletop centrifugation at high speed; remaining substrate in the isolated supernatant is quantified by HPLC-based peak detection at 280 nm. The proteolytic reaction displays first order kinetics and the rate constant, k, is determined from a plot of ln[S] versus time (k=-1 $\times$ slope).

Ex Vivo Stability Assay.

Peptidomimetic macrocycles with optimized linkers possess, for example, an ex vivo half-life that is at least two-fold greater than that of a corresponding uncrosslinked polypeptide, and possess an ex vivo half-life of 12 hours or more. For ex vivo serum stability studies, a variety of assays can be used. For example, a peptidomimetic macrocycle and a corresponding uncrosslinked polypeptide (2 mcg) are incubated with fresh mouse, rat and/or human serum (2 mL) at 37° C. for 0, 1, 2, 4, 8, and 24 hours. To determine the level of intact compound, the following procedure can be used: The samples are extracted by transferring 100 μ L of sera to 2 mL centrifuge tubes followed by the addition of 10 μ L of 50% formic acid and 500 μ L acetonitrile and centrifugation at 14,000 RPM for 10 min at 4 $\pm$ 2° C. The supernatants are then transferred to fresh 2 mL tubes and evaporated on Turbopap under N₂<10 psi, 37° C. The samples are reconstituted in 100 μ L of 50:50 acetonitrile:water and submitted to LC-MS/MS analysis.

In Vitro Binding Assays.

To assess the binding and affinity of peptidomimetic macrocycles and peptidomimetic precursors to acceptor proteins, a fluorescence polarization assay (FPA) is used, for example. The FPA technique measures the molecular orientation and mobility using polarized light and fluorescent tracer. When excited with polarized light, fluorescent tracers (e.g., FITC) attached to molecules with high apparent molecular weights (e.g. FITC-labeled peptides bound to a

large protein) emit higher levels of polarized fluorescence due to their slower rates of rotation as compared to fluorescent tracers attached to smaller molecules (e.g. FITC-labeled peptides that are free in solution).

For example, fluoresceinated peptidomimetic macrocycles (25 nM) are incubated with the acceptor protein (25-1000 nM) in binding buffer (140 mM NaCl, 50 mM Tris-HCL, pH 7.4) for 30 minutes at room temperature. Binding activity is measured, for example, by fluorescence polarization on a luminescence spectrophotometer (e.g. Perkin-Elmer LS50B). K_d values can be determined by non-linear regression analysis using, for example, Graphpad Prism software (GraphPad Software, Inc., San Diego, Calif.). A peptidomimetic macrocycle shows, in some embodiments, similar or lower K_d than a corresponding uncrosslinked polypeptide.

In Vitro Displacement Assays to Characterize Antagonists of Peptide-Protein Interactions.

To assess the binding and affinity of compounds that antagonize the interaction between a peptide and an acceptor protein, a fluorescence polarization assay (FPA) utilizing a fluoresceinated peptidomimetic macrocycle derived from a peptidomimetic precursor sequence is used, for example. The FPA technique measures the molecular orientation and mobility using polarized light and fluorescent tracer. When excited with polarized light, fluorescent tracers (e.g., FITC) attached to molecules with high apparent molecular weights (e.g. FITC-labeled peptides bound to a large protein) emit higher levels of polarized fluorescence due to their slower rates of rotation as compared to fluorescent tracers attached to smaller molecules (e.g. FITC-labeled peptides that are free in solution). A compound that antagonizes the interaction between the fluoresceinated peptidomimetic macrocycle and an acceptor protein will be detected in a competitive binding FPA experiment.

For example, putative antagonist compounds (1 nM to 1 mM) and a fluoresceinated peptidomimetic macrocycle (25 nM) are incubated with the acceptor protein (50 nM) in binding buffer (140 mM NaCl, 50 mM Tris-HCL, pH 7.4) for 30 minutes at room temperature. Antagonist binding activity is measured, for example, by fluorescence polarization on a luminescence spectrophotometer (e.g. Perkin-Elmer LS50B). K_d values can be determined by nonlinear regression analysis using, for example, Graphpad Prism software (GraphPad Software, Inc., San Diego, Calif.).

Any class of molecule, such as small organic molecules, peptides, oligonucleotides or proteins can be examined as putative antagonists in this assay.

Assay for Protein-Ligand Binding by Affinity Selection-Mass Spectrometry

To assess the binding and affinity of test compounds for proteins, an affinity-selection mass spectrometry assay is used, for example. Protein-ligand binding experiments are conducted according to the following representative procedure outlined for a system-wide control experiment using 1 μM peptidomimetic macrocycle plus 5 μM hMDM2. A 1 μL DMSO aliquot of a 40 μM stock solution of peptidomimetic macrocycle is dissolved in 19 μL of PBS (Phosphate-buffered saline: 50 mM, pH 7.5 Phosphate buffer containing 150 mM NaCl). The resulting solution is mixed by repeated pipetting and clarified by centrifugation at 10 000 g for 10 min. To a 4 μL aliquot of the resulting supernatant is added 4 μL of 10 μM hMDM2 in PBS. Each 8.0 μL experimental sample thus contains 40 pmol (1.5 μg) of protein at 5.0 μM concentration in PBS plus 1 μM peptidomimetic macrocycle and 2.5% DMSO. Duplicate samples thus prepared for each concentration point are incubated for 60 min at room tem-

perature, and then chilled to 4° C. prior to size-exclusion chromatography-LC-MS analysis of 5.0 μL injections. Samples containing a target protein, protein-ligand complexes, and unbound compounds are injected onto an SEC column, where the complexes are separated from non-binding component by a rapid SEC step. The SEC column eluate is monitored using UV detectors to confirm that the early-eluting protein fraction, which elutes in the void volume of the SEC column, is well resolved from unbound components that are retained on the column. After the peak containing the protein and protein-ligand complexes elutes from the primary UV detector, it enters a sample loop where it is excised from the flow stream of the SEC stage and transferred directly to the LC-MS via a valving mechanism. The (M+3H)³⁺ ion of the peptidomimetic macrocycle is observed by ESI-MS at the expected m/z, confirming the detection of the protein-ligand complex.

Assay for Protein-Ligand K_d Titration Experiments.

To assess the binding and affinity of test compounds for proteins, a protein-ligand K_d titration experiment is performed, for example. Protein-ligand K_d titrations experiments are conducted as follows: 2 μL DMSO aliquots of a serially diluted stock solution of titrant peptidomimetic macrocycle (5, 2.5, . . . 0.098 mM) are prepared then dissolved in 38 μL of PBS. The resulting solutions are mixed by repeated pipetting and clarified by centrifugation at 10 000 g for 10 min. To 4.0 μL aliquots of the resulting supernatants is added 4.0 μL of 10 μM hMDM2 in PBS. Each 8.0 μL experimental sample thus contains 40 pmol (1.5 μg) of protein at 5.0 μM concentration in PBS, varying concentrations (125, 62.5, . . . , 0.24 μM) of the titrant peptide, and 2.5% DMSO. Duplicate samples thus prepared for each concentration point are incubated at room temperature for 30 min, then chilled to 4° C. prior to SEC-LC-MS analysis of 2.0 μL injections. The (M+H)¹⁺, (M+2H)²⁺, (M+3H)³⁺, and/or (M+Na)¹⁺ ion is observed by ESI-MS; extracted ion chromatograms are quantified, then fit to equations to derive the binding affinity K_d as described in "A General Technique to Rank Protein-Ligand Binding Affinities and Determine Allosteric vs. Direct Binding Site Competition in Compound Mixtures." Annis, D. A.; Nazef, N.; Chuang, C. C.; Scott, M. P.; Nash, H. M. *J. Am. Chem. Soc.* 2004, 126, 15495-15503; also in "ALIS: An Affinity Selection-Mass Spectrometry System for the Discovery and Characterization of Protein-Ligand Interactions" D. A. Annis, C.-C. Chuang, and N. Nazef. In *Mass Spectrometry in Medicinal Chemistry*. Edited by Wanner K, Höfner G: Wiley-VCH; 2007:121-184. Mannhold R, Kubinyi H, Folkers G (Series Editors); *Methods and Principles in Medicinal Chemistry*.

Assay for Competitive Binding Experiments by Affinity Selection-Mass Spectrometry

To determine the ability of test compounds to bind competitively to proteins, an affinity selection mass spectrometry assay is performed, for example. A mixture of ligands at 40 μM per component is prepared by combining 2 μL aliquots of 400 μM stocks of each of the three compounds with 14 μL of DMSO. Then, 1 μL aliquots of this 40 μM per component mixture are combined with 1 μL DMSO aliquots of a serially diluted stock solution of titrant peptidomimetic macrocycle (10, 5, 2.5, . . . , 0.078 mM). These 2 μL samples are dissolved in 38 μL of PBS. The resulting solutions were mixed by repeated pipetting and clarified by centrifugation at 10 000 g for 10 min. To 4.0 μL aliquots of the resulting supernatants is added 4.0 μL of 10 μM hMDM2 protein in PBS. Each 8.0 μL experimental sample thus contains 40 pmol (1.5 μg) of protein at 5.0 μM concentration in PBS plus

0.5 μ M ligand, 2.5% DMSO, and varying concentrations (125, 62.5, . . . 0.98 μ M) of the titrant peptidomimetic macrocycle. Duplicate samples thus prepared for each concentration point are incubated at room temperature for 60 min, then chilled to 4° C. prior to SEC-LC-MS analysis of 2.0 μ L injections. Additional details on these and other methods are provided in “*A General Technique to Rank Protein-Ligand Binding Affinities and Determine Allosteric vs. Direct Binding Site Competition in Compound Mixtures.*” Annis, D. A.; Nazef, N.; Chuang, C. C.; Scott, M. P.; Nash, H. M. J. Am. Chem. Soc. 2004, 126, 15495-15503; also in “*ALIS: An Affinity Selection-Mass Spectrometry System for the Discovery and Characterization of Protein-Ligand Interactions.*” D. A. Annis, C.-C. Chuang, and N. Nazef. In Mass Spectrometry in Medicinal Chemistry. Edited by Wanner K, Höfner G: Wiley-VCH; 2007:121-184. Mannhold R, Kubinyi H, Folkers G (Series Editors): Methods and Principles in Medicinal Chemistry.

Binding Assays in Intact Cells.

It is possible to measure binding of peptides or peptidomimetic macrocycles to their natural acceptors in intact cells by immunoprecipitation experiments. For example, intact cells are incubated with fluoresceinated (FITC-labeled) compounds for 4 hrs in the absence of serum, followed by serum replacement and further incubation that ranges from 4-18 hrs. Cells are then pelleted and incubated in lysis buffer (50 mM Tris [pH 7.6], 150 mM NaCl, 1% CHAPS and protease inhibitor cocktail) for 10 minutes at 4° C. Extracts are centrifuged at 14,000 rpm for 15 minutes and supernatants collected and incubated with 10 μ L goat anti-FITC antibody for 2 hrs, rotating at 4° C. followed by further 2 hrs incubation at 4° C. with protein A/G Sepharose (50 μ L of 50% bead slurry). After quick centrifugation, the pellets are washed in lysis buffer containing increasing salt concentration (e.g., 150, 300, 500 mM). The beads are then re-equilibrated at 150 mM NaCl before addition of SDS-containing sample buffer and boiling. After centrifugation, the supernatants are optionally electrophoresed using 4%-12% gradient Bis-Tris gels followed by transfer into Immobilon-P membranes. After blocking, blots are optionally incubated with an antibody that detects FITC and also with one or more antibodies that detect proteins that bind to the peptidomimetic macrocycle.

Cellular Penetrability Assays.

A peptidomimetic macrocycle is, for example, more cell penetrable compared to a corresponding uncrosslinked macrocycle. Peptidomimetic macrocycles with optimized linkers possess, for example, cell penetrability that is at least two-fold greater than a corresponding uncrosslinked macrocycle, and often 20% or more of the applied peptidomimetic macrocycle will be observed to have penetrated the cell after 4 hours. To measure the cell penetrability of peptidomimetic macrocycles and corresponding uncrosslinked macrocycle, intact cells are incubated with fluorescently-labeled (e.g. fluoresceinated) peptidomimetic macrocycles or corresponding uncrosslinked macrocycle (10 μ M) for 4 hrs in serum free media at 37° C., washed twice with media and incubated with trypsin (0.25%) for 10 min at 37° C. The cells are washed again and resuspended in PBS. Cellular fluorescence is analyzed, for example, by using either a FACSCalibur flow cytometer or Cellomics' KineticScan® HCS Reader.

Cellular Efficacy Assays.

The efficacy of certain peptidomimetic macrocycles is determined, for example, in cell-based killing assays using a variety of tumorigenic and non-tumorigenic cell lines and primary cells derived from human or mouse cell popula-

tions. Cell viability is monitored, for example, over 24-96 hrs of incubation with peptidomimetic macrocycles (0.5 to 50 μ M) to identify those that kill at $EC_{50} < 10 \mu$ M. Several standard assays that measure cell viability are commercially available and are optionally used to assess the efficacy of the peptidomimetic macrocycles. In addition, assays that measure Annexin V and caspase activation are optionally used to assess whether the peptidomimetic macrocycles kill cells by activating the apoptotic machinery. For example, the Cell Titer-glo assay is used which determines cell viability as a function of intracellular ATP concentration.

In Vivo Stability Assay.

To investigate the in vivo stability of the peptidomimetic macrocycles, the compounds are, for example, administered to mice and/or rats by IV, IP, PO or inhalation routes at concentrations ranging from 0.1 to 50 mg/kg and blood specimens withdrawn at 0', 5', 15', 30', 1 hr, 4 hrs, 8 hrs and 24 hours post-injection. Levels of intact compound in 25 μ L of fresh serum are then measured by LC-MS/MS as above.

In Vivo Efficacy in Animal Models.

To determine the anti-oncogenic activity of peptidomimetic macrocycles in vivo, the compounds are, for example, given alone (IP, IV, PO, by inhalation or nasal routes) or in combination with sub-optimal doses of relevant chemotherapy (e.g., cyclophosphamide, doxorubicin, etoposide). In one example, 5×10^6 RS4; 11 cells (established from the bone marrow of a patient with acute lymphoblastic leukemia) that stably express luciferase are injected by tail vein in NOD-SCID mice 3 hrs after they have been subjected to total body irradiation. If left untreated, this form of leukemia is fatal in 3 weeks in this model. The leukemia is readily monitored, for example, by injecting the mice with D-luciferin (60 mg/kg) and imaging the anesthetized animals (e.g., Xenogen In Vivo Imaging System, Caliper Life Sciences, Hopkinton, Mass.). Total body bioluminescence is quantified by integration of photonic flux (photons/sec) by Living Image Software (Caliper Life Sciences, Hopkinton, Mass.). Peptidomimetic macrocycles alone or in combination with sub-optimal doses of relevant chemotherapeutics agents are, for example, administered to leukemic mice (10 days after injection/day 1 of experiment, in bioluminescence range of 14-16) by tail vein or IP routes at doses ranging from 0.1 mg/kg to 50 mg/kg for 7 to 21 days. Optionally, the mice are imaged throughout the experiment every other day and survival monitored daily for the duration of the experiment. Expired mice are optionally subjected to necropsy at the end of the experiment. Another animal model is implantation into NOD-SCID mice of DoHH2, a cell line derived from human follicular lymphoma, that stably expresses luciferase. These in vivo tests optionally generate preliminary pharmacokinetic, pharmacodynamic and toxicology data.

Clinical Trials.

To determine the suitability of the peptidomimetic macrocycles for treatment of humans, clinical trials are performed. For example, patients diagnosed with cancer and in need of treatment can be selected and separated in treatment and one or more control groups, wherein the treatment group is administered a peptidomimetic macrocycle, while the control groups receive a placebo or a known anti-cancer drug. The treatment safety and efficacy of the peptidomimetic macrocycles can thus be evaluated by performing comparisons of the patient groups with respect to factors such as survival and quality-of-life. In this example, the patient group treated with a peptidomimetic macrocycle can show improved long-term survival compared to a patient control group treated with a placebo.

Pharmaceutical Compositions and Routes of Administration

Pharmaceutical compositions disclosed herein include peptidomimetic macrocycles and pharmaceutically acceptable derivatives or prodrugs thereof. A "pharmaceutically acceptable derivative" means any pharmaceutically acceptable salt, ester, salt of an ester, pro-drug or other derivative of a compound disclosed herein which, upon administration to a recipient, is capable of providing (directly or indirectly) a compound disclosed herein. Particularly favored pharmaceutically acceptable derivatives are those that increase the bioavailability of the compounds when administered to a mammal (e.g., by increasing absorption into the blood of an orally administered compound) or which increases delivery of the active compound to a biological compartment (e.g., the brain or lymphatic system) relative to the parent species. Some pharmaceutically acceptable derivatives include a chemical group which increases aqueous solubility or active transport across the gastrointestinal mucosa.

In some embodiments, peptidomimetic macrocycles are modified by covalently or non-covalently joining appropriate functional groups to enhance selective biological properties. Such modifications include those which increase biological penetration into a given biological compartment (e.g., blood, lymphatic system, central nervous system), increase oral availability, increase solubility to allow administration by injection, alter metabolism, and alter rate of excretion.

Pharmaceutically acceptable salts of the compounds disclosed herein include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acid salts include acetate, adipate, benzoate, benzenesulfonate, butyrate, citrate, digluconate, dodecylsulfate, formate, fumarate, glycolate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, lactate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, palmoate, phosphate, picrate, pivalate, propionate, salicylate, succinate, sulfate, tartrate, tosylate and undecanoate. Salts derived from appropriate bases include alkali metal (e.g., sodium), alkaline earth metal (e.g., magnesium), ammonium and N-(alkyl)₄⁺ salts.

For preparing pharmaceutical compositions from the compounds disclosed herein, pharmaceutically acceptable carriers include either solid or liquid carriers. Solid form preparations include powders, tablets, pills, capsules, cachets, suppositories, and dispersible granules. A solid carrier can be one or more substances, which also acts as diluents, flavoring agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material. Details on techniques for formulation and administration are well described in the scientific and patent literature, see, e.g., the latest edition of Remington's Pharmaceutical Sciences, Maack Publishing Co, Easton Pa.

In powders, the carrier is a finely divided solid, which is in a mixture with the finely divided active component. In tablets, the active component is mixed with the carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired.

Suitable solid excipients are carbohydrate or protein fillers include, but are not limited to sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethyl-cellulose; and gums including arabic and tragacanth; as well as proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents are added, such as the cross-

linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Liquid form preparations include solutions, suspensions, and emulsions, for example, water or water/propylene glycol solutions. For parenteral injection, liquid preparations can be formulated in solution in aqueous polyethylene glycol solution.

The pharmaceutical preparation can be in unit dosage form. In such form the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form.

When one or more compositions disclosed herein comprise a combination of a peptidomimetic macrocycle and one or more additional therapeutic or prophylactic agents, both the compound and the additional agent should be present at dosage levels of between about 1 to 100%, and more preferably between about 5 to 95% of the dosage normally administered in a monotherapy regimen. In some embodiments, the additional agents are administered separately, as part of a multiple dose regimen, from one or more compounds disclosed herein. Alternatively, those agents are part of a single dosage form, mixed together with the compounds disclosed herein in a single composition.

Methods of Use

In one aspect, provided herein are novel peptidomimetic macrocycles that are useful in competitive binding assays to identify agents which bind to the natural ligand(s) of the proteins or peptides upon which the peptidomimetic macrocycles are modeled. For example, in the p53/MDMX system, labeled peptidomimetic macrocycles based on p53 can be used in a MDMX binding assay along with small molecules that competitively bind to MDMX. Competitive binding studies allow for rapid in vitro evaluation and determination of drug candidates specific for the p53/MDMX system. Such binding studies can be performed with any of the peptidomimetic macrocycles disclosed herein and their binding partners.

Further provided are methods for the generation of antibodies against the peptidomimetic macrocycles. In some embodiments, these antibodies specifically bind both the peptidomimetic macrocycle and the precursor peptides, such as p53, to which the peptidomimetic macrocycles are related. Such antibodies, for example, disrupt the native protein-protein interaction, for example, binding between p53 and MDMX.

In other aspects, provided herein are both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant (e.g., insufficient or excessive) expression or activity of the molecules including p53, MDM2 or MDMX.

In another embodiment, a disorder is caused, at least in part, by an abnormal level of p53 or MDM2 or MDMX, (e.g., over or under expression), or by the presence of p53 or MDM2 or MDMX exhibiting abnormal activity. As such, the reduction in the level and/or activity of p53 or MDM2 or MDMX, or the enhancement of the level and/or activity of p53 or MDM2 or MDMX, by peptidomimetic macrocycles derived from p53, is used, for example, to ameliorate or reduce the adverse symptoms of the disorder.

In another aspect, provided herein are methods for treating or preventing a disease including hyperproliferative disease and inflammatory disorder by interfering with the

interaction or binding between binding partners, for example, between p53 and MDM2 or p53 and MDMX. These methods comprise administering an effective amount of a compound to a warm blooded animal, including a human. In some embodiments, the administration of one or more compounds disclosed herein induces cell growth arrest or apoptosis.

As used herein, the term "treatment" is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease, the symptoms of disease or the predisposition toward disease.

In some embodiments, the peptidomimetic macrocycles can be used to treat, prevent, and/or diagnose cancers and neoplastic conditions. As used herein, the terms "cancer", "hyperproliferative" and "neoplastic" refer to cells having the capacity for autonomous growth, i.e., an abnormal state or condition characterized by rapidly proliferating cell growth. Hyperproliferative and neoplastic disease states can be categorized as pathologic, i.e., characterizing or constituting a disease state, or can be categorized as non-pathologic, i.e., a deviation from normal but not associated with a disease state. The term is meant to include all types of cancerous growths or oncogenic processes, metastatic tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. A metastatic tumor can arise from a multitude of primary tumor types, including but not limited to those of breast, lung, liver, colon and ovarian origin. "Pathologic hyperproliferative" cells occur in disease states characterized by malignant tumor growth. Examples of non-pathologic hyperproliferative cells include proliferation of cells associated with wound repair. Examples of cellular proliferative and/or differentiative disorders include cancer, e.g., carcinoma, sarcoma, or metastatic disorders. In some embodiments, the peptidomimetic macrocycles are novel therapeutic agents for controlling breast cancer, ovarian cancer, colon cancer, lung cancer, metastasis of such cancers and the like.

Examples of cancers or neoplastic conditions include, but are not limited to, a fibrosarcoma, myosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, gastric cancer, esophageal cancer, rectal cancer, pancreatic cancer, ovarian cancer, prostate cancer, uterine cancer, cancer of the head and neck, skin cancer, brain cancer, squamous cell carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinoma, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular cancer, small cell lung carcinoma, non-small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma, leukemia, lymphoma, or Kaposi sarcoma.

In some embodiments, the cancer is head and neck cancer, melanoma, lung cancer, breast cancer, or glioma.

Examples of proliferative disorders include hematopoietic neoplastic disorders. As used herein, the term "hematopoietic neoplastic disorders" includes diseases involving hyper-

plastic/neoplastic cells of hematopoietic origin, e.g., arising from myeloid, lymphoid or erythroid lineages, or precursor cells thereof. The diseases can arise from poorly differentiated acute leukemias, e.g., erythroblastic leukemia and acute megakaryoblastic leukemia. Additional exemplary myeloid disorders include, but are not limited to, acute promyeloid leukemia (APML), acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML) (reviewed in Vaickus (1991), *Crit. Rev. Oncol./Hematol.* 11:267-97); lymphoid malignancies include, but are not limited to acute lymphoblastic leukemia (ALL) which includes B-lineage ALL and T-lineage ALL, chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), hairy cell leukemia (HLL) and Waldenstrom's macroglobulinemia (WM). Additional forms of malignant lymphomas include, but are not limited to non-Hodgkin lymphoma and variants thereof, peripheral T cell lymphomas, adult T cell leukemia/lymphoma (ATL), cutaneous T-cell lymphoma (CTCL), large granular lymphocytic leukemia (LGL), Hodgkin's disease and Reed-Stemberg disease.

Examples of cellular proliferative and/or differentiative disorders of the breast include, but are not limited to, proliferative breast disease including, e.g., epithelial hyperplasia, sclerosing adenosis, and small duct papillomas; tumors, e.g., stromal tumors such as fibroadenoma, phyllodes tumor, and sarcomas, and epithelial tumors such as large duct papilloma; carcinoma of the breast including in situ (noninvasive) carcinoma that includes ductal carcinoma in situ (including Paget's disease) and lobular carcinoma in situ, and invasive (infiltrating) carcinoma including, but not limited to, invasive ductal carcinoma, invasive lobular carcinoma, medullary carcinoma, colloid (mucinous) carcinoma, tubular carcinoma, and invasive papillary carcinoma, and miscellaneous malignant neoplasms. Disorders in the male breast include, but are not limited to, gynecomastia and carcinoma.

Examples of cellular proliferative and/or differentiative disorders of the skin include, but are not limited to proliferative skin disease such as melanomas, including mucosal melanoma, superficial spreading melanoma, nodular melanoma, lentigo (e.g. lentigo maligna, lentigo maligna melanoma, or acral lentiginous melanoma), amelanotic melanoma, desmoplastic melanoma, melanoma with features of a Spitz nevus, melanoma with small nevus-like cells, polypoid melanoma, and soft-tissue melanoma; basal cell carcinomas including micronodular basal cell carcinoma, superficial basal cell carcinoma, nodular basal cell carcinoma (rodent ulcer), cystic basal cell carcinoma, cicatricial basal cell carcinoma, pigmented basal cell carcinoma, aberrant basal cell carcinoma, infiltrative basal cell carcinoma, nevoid basal cell carcinoma syndrome, polypoid basal cell carcinoma, pore-like basal cell carcinoma, and fibroepithelioma of Pinkus; squamous cell carcinomas including acanthoma (large cell acanthoma), adenoid squamous cell carcinoma, basaloid squamous cell carcinoma, clear cell squamous cell carcinoma, signet-ring cell squamous cell carcinoma, spindle cell squamous cell carcinoma, Marjolin's ulcer, erythroplasia of Queyrat, and Bowen's disease; or other skin or subcutaneous tumors.

Examples of cellular proliferative and/or differentiative disorders of the lung include, but are not limited to, bronchogenic carcinoma, including paraneoplastic syndromes, bronchioloalveolar carcinoma, neuroendocrine tumors, such as bronchial carcinoid, miscellaneous tumors, and metastatic tumors; pathologies of the pleura, including inflammatory pleural effusions, noninflammatory pleural effusions, pneu-

57

mothorax, and pleural tumors, including solitary fibrous tumors (pleural fibroma) and malignant mesothelioma.

Examples of cellular proliferative and/or differentiative disorders of the colon include, but are not limited to, non-neoplastic polyps, adenomas, familial syndromes, colorectal carcinogenesis, colorectal carcinoma, and carcinoid tumors.

Examples of cellular proliferative and/or differentiative disorders of the liver include, but are not limited to, nodular hyperplasias, adenomas, and malignant tumors, including primary carcinoma of the liver and metastatic tumors.

Examples of cellular proliferative and/or differentiative disorders of the ovary include, but are not limited to, ovarian tumors such as, tumors of coelomic epithelium, serous tumors, mucinous tumors, endometrioid tumors, clear cell adenocarcinoma, cystadenofibroma, Brenner tumor, surface epithelial tumors; germ cell tumors such as mature (benign) teratomas, monodermal teratomas, immature malignant teratomas, dysgerminoma, endodermal sinus tumor, choriocarcinoma; sex cord-stomal tumors such as, granulosa-theca

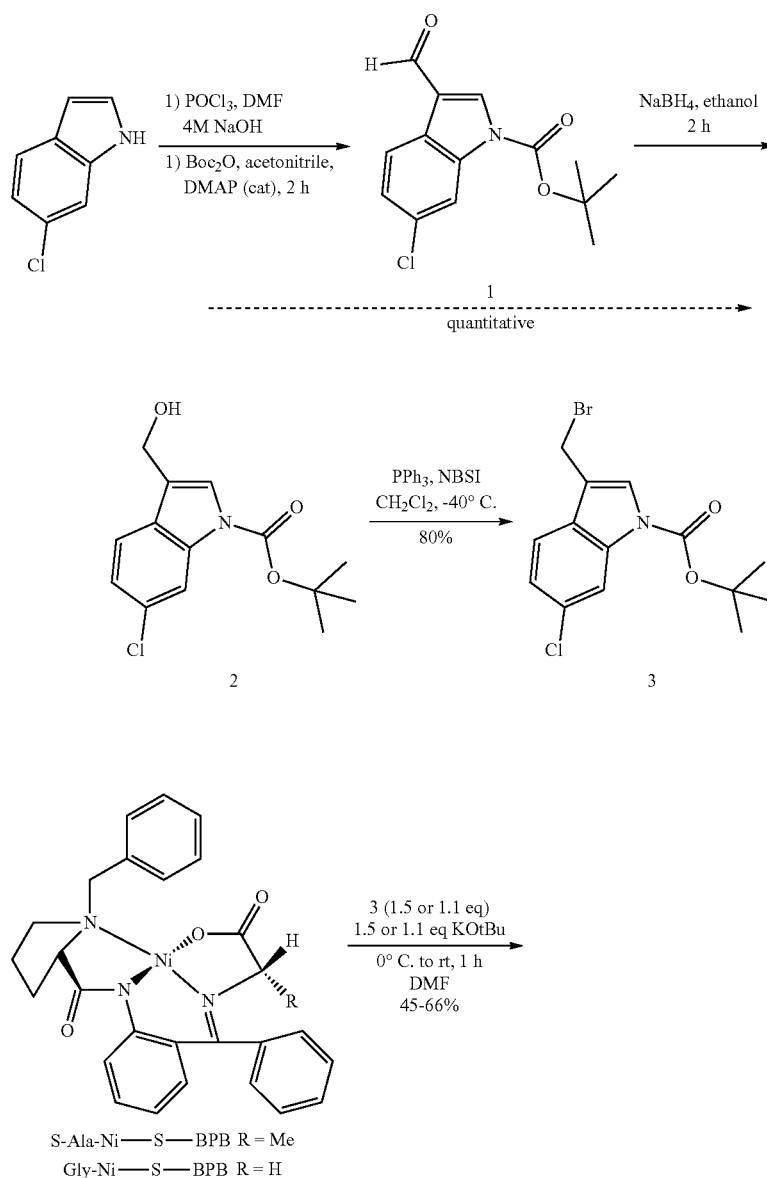
58

cell tumors, thecomafibromas, androblastomas, hill cell tumors, and gonadoblastoma; and metastatic tumors such as Krukenberg tumors.

While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments described herein can be employed in practicing the invention. It is intended that the following claims define the scope and that methods and structures within the scope of these claims and their equivalents be covered thereby.

EXAMPLES

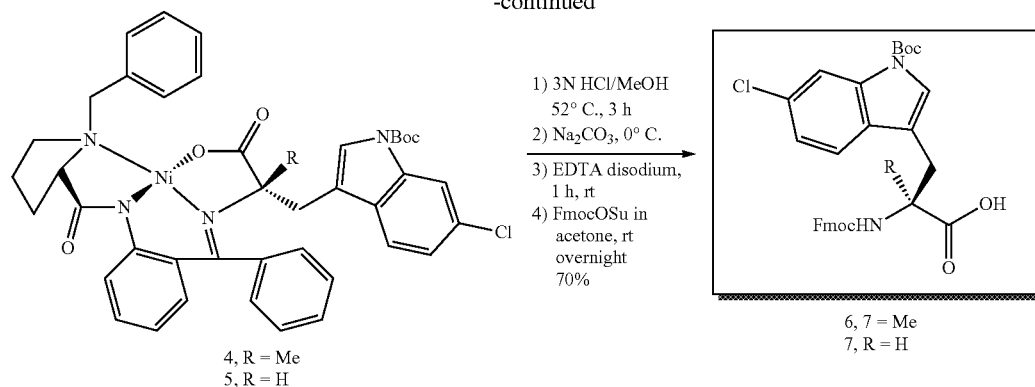
Example 1: Synthesis of 6-Chlorotryptophan Fmoc Amino Acids



59

60

-continued



Tert-butyl 6-chloro-3-formyl-1H-indole-1-carboxylate, 1. To a stirred solution of dry DMF (12 mL) was added dropwise POCl₃ (3.92 mL, 43 mmol, 1.3 equiv) at 0° C. under Argon. The solution was stirred at the same temperature for 20 min before a solution of 6-chloroindole (5.0 g, 33 mmol, 1 eq.) in dry DMF (30 mL) was added dropwise. The resulting mixture was allowed to warm to room temperature and stirred for an additional 2.5 h. Water (50 mL) was added and the solution was neutralized with 4 μM aqueous NaOH (pH~8). The resulting solid was filtered off, washed with water and dried under vacuum. This material was directly used in the next step without additional purification. To a stirred solution of the crude formyl indole (33 mmol, 1 eq.) in THF (150 mL) was added successively Boc₂O (7.91 g, 36.3 mmol, 1.1 equiv) and DMAP (0.4 g, 3.3 mmol, 0.1 equiv) at room temperature under N₂. The resulting mixture was stirred at room temperature for 1.5 h and the solvent was evaporated under reduced pressure. The residue was taken up in EtOAc and washed with 1N HCl, dried and concentrated to give the formyl indole 1 (9 g, 98% over 2 steps) as a white solid. ¹H NMR (CDCl₃) δ: 1.70 (s, Boc, 9H); 7.35 (dd, 1H); 8.21 (m, 3H); 10.07 (s, 1H).

Tert-butyl 6-chloro-3-(hydroxymethyl)-1H-indole-1-carboxylate, 2. To a solution of compound 1 (8.86 g, 32 mmol, 1 eq.) in ethanol (150 mL) was added NaBH₄ (2.4 g, 63 mmol, 2 eq.). The reaction was stirred for 3 h at room temperature. The reaction mixture was concentrated and the residue was poured into diethyl ether and water. The organic layer was separated, dried over magnesium sulfate and concentrated to give a white solid (8.7 g, 98%). This material was directly used in the next step without additional purification. ¹H NMR (CDCl₃) δ: 1.65 (s, Boc, 9H); 4.80 (s, 2H, CH₂); 7.21 (dd, 1H); 7.53 (m, 2H); 8.16 (bs, 1H).

Tert-butyl 3-(bromomethyl)-6-chloro-1H-indole-1-carboxylate, 3. To a solution of compound 2 (4.1 g, 14.6 mmol, 1 eq.) in dichloromethane (50 mL) under argon was added a solution of triphenylphosphine (4.59 g, 17.5 mmol, 1.2 eq.) in dichloromethane (50 mL) at -40° C. The reaction solution was stirred an additional 30 min at 40° C. Then NBS (3.38 g, 19 mmol, 1.3 eq.) was added. The resulting mixture was allowed to warm to room temperature and stirred overnight. Dichloromethane was evaporated, Carbon Tetrachloride (100 mL) was added and the mixture was stirred for 1 h and filtrated. The filtrate was concentrated, loaded in a silica plug and quickly eluted with 25% EtOAc in Hexanes. The solution was concentrated to give a white foam (3.84 g, 77%). ¹H NMR (CDCl₃) δ: 1.66 (s, Boc, 9H); 4.63 (s, 2H, CH₂); 7.28 (dd, 1H); 7.57 (d, 1H); 7.64 (bs, 1H); 8.18 (bs, 1H).

αMe-6Cl-Trp(Boc)-Ni—S—BPB, 4. To S-Ala-Ni—S—BPB (2.66 g, 5.2 mmol, 1 eq.) and KO-tBu (0.87 g, 7.8 mmol, 1.5 eq.) was added 50 mL of DMF under argon. The bromide derivative compound 3 (2.68 g, 7.8 mmol, 1.5 eq.) in solution of DMF (5.0 mL) was added via syringe. The reaction mixture was stirred at ambient temperature for 1 h. The solution was then quenched with 5% aqueous acetic acid and diluted with water. The desired product was extracted in dichloromethane, dried and concentrated. The oily product 4 was purified by flash chromatography (solid loading) on normal phase using EtOAc and Hexanes as eluents to give a red solid (1.78 g, 45% yield). αMe-6Cl-Trp(Boc)-Ni—S—BPB, 4: M+H calc. 775.21, M+H obs. 775.26; ¹H NMR (CDCl₃) δ: 1.23 (s, 3H, αMe); 1.56 (m, 1H, Boc+CH₂); 1.82-2.20 (m, 4H, 2CH₂); 3.03 (m, 1H, CH₂); 3.24 (m, 2H, CH₂); 3.57 and 4.29 (AB system, 2H, CH₂ (benzyl), J=12.8 Hz); 6.62 (d, 2H); 6.98 (d, 1H); 7.14 (m, 2H); 7.23 (m, 1H); 7.32-7.36 (m, 5H); 7.50 (m, 2H); 7.67 (bs, 1H); 7.98 (d, 2H); 8.27 (m, 2H).

Fmoc-αMe-6Cl-Trp(Boc)-OH, 6. To a solution of 3N HCl/MeOH (1/3, 15 mL) at 50° C. was added a solution of compound 4 (1.75 g, 2.3 mmol, 1 eq.) in MeOH (5 mL) dropwise. The starting material disappeared within 3-4 h. The acidic solution was then cooled to 0° C. with an ice bath and quenched with an aqueous solution of Na₂CO₃ (1.21 g, 11.5 mmol, 5 eq.). Methanol was removed and 8 more equivalents of Na₂CO₃ (1.95 g, 18.4 mmol) were added to the suspension. The Nickel scavenging EDTA disodium salt dihydrate (1.68 g, 4.5 mmol, 2 eq.) was then added and the suspension was stirred for 2 h. A solution of Fmoc-OSu (0.84 g, 2.5 mmol, 1.1 eq.) in acetone (50 mL) was added and the reaction was stirred overnight. Afterwards, the reaction was diluted with diethyl ether and 1N HCl. The organic layer was then dried over magnesium sulfate and concentrated in vacuo. The desired product 6 was purified on normal phase using acetone and dichloromethane as eluents to give a white foam (0.9 g, 70% yield). Fmoc-αMe-6Cl-Trp(Boc)-OH, 6: M+H calc. 575.19, M+H obs. 575.37; ¹H NMR (CDCl₃) δ: 1.59 (s, 9H, Boc); 1.68 (s, 3H, Me); 3.48 (bs, 2H, CH₂); 4.22 (m, 1H, CH); 4.39 (bs, 2H, CH₂); 5.47 (s, 1H, NH); 7.10 (m, 1H); 7.18 (m, 2H); 7.27 (m, 2H); 7.39 (m, 2H); 7.50 (m, 2H); 7.75 (d, 2H); 8.12 (bs, 1H).

6Cl-Trp(Boc)-Ni—S—BPB, 5. To Gly-Ni—S—BPB (4.6 g, 9.2 mmol, 1 eq.) and KO-tBu (1.14 g, 10.1 mmol, 1.1 eq.) was added 95 mL of DMF under argon. The bromide derivative compound 3 (3.5 g, 4.6 mmol, 1.1 eq.) in solution of DMF (10 mL) was added via syringe. The reaction mixture was stirred at ambient temperature for 1 h. The solution was then quenched with 5% aqueous acetic acid and

61

diluted with water. The desired product was extracted in dichloromethane, dried and concentrated. The oily product 5 was purified by flash chromatography (solid loading) on normal phase using EtOAc and Hexanes as eluents to give a red solid (5 g, 71% yield). 6Cl-Trp(Boc)-Ni—S—BPPB, 5: M+H calc. 761.20, M+H obs. 761.34; ¹H NMR (CDCl₃) δ: 1.58 (m, 11H, Boc+CH₂); 1.84 (m, 1H); 1.96 (m, 1H); 2.24 (m, 2H, CH₂); 3.00 (m, 1H, CH_α); 3.22 (m, 2H, CH₂); 3.45 and 4.25 (AB system, 2H, CH₂ (benzyl), J=12.8 Hz); 4.27 (m, 1H, CH_α); 6.65 (d, 2H); 6.88 (d, 1H); 7.07 (m, 2H); 7.14 (m, 2H); 7.28 (m, 3H); 7.35-7.39 (m, 2H); 7.52 (m, 2H); 7.96 (d, 2H); 8.28 (m, 2H).

Fmoc-6Cl-Trp(Boc)-OH, 7. To a solution of 3N HCl/MeOH (1/3, 44 mL) at 50° C. was added a solution of compound 5 (5 g, 6.6 mmol, 1 eq.) in MeOH (10 ml) dropwise. The starting material disappeared within 3-4 h. The acidic solution was then cooled to 0° C. with an ice bath and quenched with an aqueous solution of Na₂CO₃ (3.48 g, 33 mmol, 5 eq.). Methanol was removed and 8 more equivalents of Na₂CO₃ (5.57 g, 52 mmol) were added to the suspension. The Nickel scavenging EDTA disodium salt dihydrate (4.89 g, 13.1 mmol, 2 eq.) and the suspension was stirred for 2 h. A solution of Fmoc-OSu (2.21 g, 6.55 mmol, 1.1 eq.) in acetone (100 mL) was added and the reaction was stirred overnight. Afterwards, the reaction was diluted with diethyl ether and 1N HCl. The organic layer was then dried over magnesium sulfate and concentrated in vacuo. The desired product 7 was purified on normal phase using acetone and dichloromethane as eluents to give a white foam (2.6 g, 69% yield). Fmoc-6Cl-Trp(Boc)-OH, 7: M+H calc. 561.17, M+H obs. 561.37; ¹H NMR (CDCl₃) δ: 1.63 (s, 9H, Boc); 3.26 (m, 2H, CH₂); 4.19 (m, 1H, CH); 4.39 (m, 2H, CH₂); 4.76 (m, 1H); 5.35 (d, 1H, NH); 7.18 (m, 2H); 7.28 (m, 2H); 7.39 (m, 3H); 7.50 (m, 2H); 7.75 (d, 2H); 8.14 (bs, 1H).

Example 2: Peptidomimetic Macrocycles

Peptidomimetic macrocycles were synthesized, purified and analyzed as previously described and as described below (Schafmeister et al., J. Am. Chem. Soc. 122:5891-5892 (2000); Schafmeister & Verdine, J. Am. Chem. Soc. 122:5891 (2005); Walensky et al., Science 305:1466-1470 (2004); and U.S. Pat. No. 7,192,713). Peptidomimetic macrocycles were designed by replacing two or more naturally occurring amino acids with the corresponding synthetic amino acids. Substitutions were made at i and i+4, and i and i+7 positions. Peptide synthesis was performed either manually or on an automated peptide synthesizer (Applied Biosystems, model 433A), using solid phase conditions, rink amide AM resin (Novabiochem), and Fmoc main-chain protecting group chemistry. For the coupling of natural Fmoc-protected amino acids (Novabiochem), 10 equivalents of amino acid and a 1:1:2 molar ratio of coupling reagents HBTU/HOBt (Novabiochem)/DIEA were employed. Non-natural amino acids (4 equiv) were coupled with a 1:1:2 molar ratio of HATU (Applied Biosystems)/HOBt/DIEA. The N-termini of the synthetic peptides were acetylated, while the C-termini were amidated.

Purification of cross-linked compounds was achieved by high performance liquid chromatography (HPLC) (Varian ProStar) on a reverse phase C18 column (Varian) to yield the pure compounds. Chemical composition of the pure products was confirmed by LC/MS mass spectrometry (Micro-mass LCT interfaced with Agilent 1100 HPLC system) and amino acid analysis (Applied Biosystems, model 420A).

62

The following protocol was used in the synthesis of dialkyne-crosslinked peptidomimetic macrocycles, including SP662, SP663 and SP664. Fully protected resin-bound peptides were synthesized on a PEG-PS resin (loading 0.45 mmol/g) on a 0.2 mmol scale. Deprotection of the temporary Fmoc group was achieved by 3×10 min treatments of the resin bound peptide with 20% (v/v) piperidine in DMF. After washing with NMP (3×), dichloromethane (3×) and NMP (3×), coupling of each successive amino acid was achieved with 1×60 min incubation with the appropriate preactivated Fmoc-amino acid derivative. All protected amino acids (0.4 mmol) were dissolved in NMP and activated with HCTU (0.4 mmol) and DIEA (0.8 mmol) prior to transfer of the coupling solution to the deprotected resin-bound peptide. After coupling was completed, the resin was washed in preparation for the next deprotection/coupling cycle. Acetylation of the amino terminus was carried out in the presence of acetic anhydride/DIEA in NMP. The LC-MS analysis of a cleaved and deprotected sample obtained from an aliquot of the fully assembled resin-bound peptide was accomplished in order to verifying the completion of each coupling. In a typical example, tetrahydrofuran (4 ml) and triethylamine (2 ml) were added to the peptide resin (0.2 mmol) in a 40 ml glass vial and shaken for 10 minutes. Pd(PPh₃)₂Cl₂ (0.014 g, 0.02 mmol) and copper iodide (0.008 g, 0.04 mmol) were then added and the resulting reaction mixture was mechanically shaken 16 hours while open to atmosphere. The diyne-cyclized resin-bound peptides were deprotected and cleaved from the solid support by treatment with TFA/H₂O/TIS (95/5/5 v/v) for 2.5 h at room temperature. After filtration of the resin the TFA solution was precipitated in cold diethyl ether and centrifuged to yield the desired product as a solid. The crude product was purified by preparative HPLC.

The following protocol was used in the synthesis of single alkyne-crosslinked peptidomimetic macrocycles, including SP665. Fully protected resin-bound peptides were synthesized on a Rink amide MBHA resin (loading 0.62 mmol/g) on a 0.1 mmol scale. Deprotection of the temporary Fmoc group was achieved by 2×20 min treatments of the resin bound peptide with 25% (v/v) piperidine in NMP. After extensive flow washing with NMP and dichloromethane, coupling of each successive amino acid was achieved with 1×60 min incubation with the appropriate preactivated Fmoc-amino acid derivative. All protected amino acids (1 mmol) were dissolved in NMP and activated with HCTU (1 mmol) and DIEA (1 mmol) prior to transfer of the coupling solution to the deprotected resin-bound peptide. After coupling was completed, the resin was extensively flow washed in preparation for the next deprotection/coupling cycle. Acetylation of the amino terminus was carried out in the presence of acetic anhydride/DIEA in NMP/NMM. The LC-MS analysis of a cleaved and deprotected sample obtained from an aliquot of the fully assembled resin-bound peptide was accomplished in order to verifying the completion of each coupling. In a typical example, the peptide resin (0.1 mmol) was washed with DCM. Resin was loaded into a microwave vial. The vessel was evacuated and purged with nitrogen. Molybdenumhexacarbonyl (0.01 eq, Sigma Aldrich 199959) was added. Anhydrous chlorobenzene was added to the reaction vessel. Then 2-fluorophenol (1 eq, Sigma Aldrich F12804) was added. The reaction was then loaded into the microwave and held at 130° C. for 10 minutes. Reaction may need to be pushed a subsequent time for completion. The alkyne metathesized resin-bound peptides were deprotected and cleaved from the solid support by treatment with TFA/H₂O/TIS (94/3/3 v/v) for 3 h at room temperature. After filtration of the resin the TFA solution was precipitated in cold diethyl ether and centrifuged to yield the desired product as a solid. The crude product was purified by preparative HPLC.

Table 1 shows a list of peptidomimetic macrocycles prepared.

TABLE 1

SP	Sequence	SEQ ID NO:	Isomer	Exact Mass	Found Mass	Calc (M + 1)/1	Calc (M + 2)/2	Calc (M + 3)/3
SP1	Ac-F\$8AYWEAc3cL\$AAA-NH2	10		1456.78	729.44	1457.79	729.4	486.6
SP2	Ac-F\$8AYWEAc3cL\$AAibA-NH2	11		1470.79	736.4	1471.8	736.4	491.27
SP3	Ac-LTF\$8AYWAQL\$SSANle-NH2	12		1715.97	859.02	1716.98	858.99	573
SP4	Ac-LTF\$8AYWAQL\$SAL-NH2	13		1715.97	859.02	1716.98	858.99	573
SP5	Ac-LTF\$8AYWAQL\$SAM-NH2	14		1733.92	868.48	1734.93	867.97	578.98
SP6	Ac-LTF\$8AYWAQL\$SAhL-NH2	15		1729.98	865.98	1730.99	866	577.67
SP7	Ac-LTF\$8AYWAQL\$SAF-NH2	16		1749.95	876.36	1750.96	875.98	584.32
SP8	Ac-LTF\$8AYWAQL\$SAI-NH2	17		1715.97	859.02	1716.98	858.99	573
SP9	Ac-LTF\$8AYWAQL\$SAChg-NH2	18		1741.98	871.98	1742.99	872	581.67
SP10	Ac-LTF\$8AYWAQL\$SAAib-NH2	19		1687.93	845.36	1688.94	844.97	563.65
SP11	Ac-LTF\$8AYWAQL\$SAA-NH2	20		1673.92	838.01	1674.93	837.97	558.98
SP12	Ac-LTF\$8AYWASL\$SSNle-NH2	21		1767.04	884.77	1768.05	884.53	590.02
SP13	Ac-LTF\$8AYWASL\$SSA-NH2	22		1724.99	864.23	1726	863.5	576
SP14	Ac-F\$8AYWEAc3cL\$AANle-NH2	23		1498.82	750.46	1499.83	750.42	500.61
SP15	Ac-F\$8AYWEAc3cL\$AAL-NH2	24		1498.82	750.46	1499.83	750.42	500.61
SP16	Ac-F\$8AYWEAc3cL\$AAM-NH2	25		1516.78	759.41	1517.79	759.4	506.6
SP17	Ac-F\$8AYWEAc3cL\$AAhL-NH2	26		1512.84	757.49	1513.85	757.43	505.29
SP18	Ac-F\$8AYWEAc3cL\$AAF-NH2	27		1532.81	767.48	1533.82	767.41	511.94
SP19	Ac-F\$8AYWEAc3cL\$AAI-NH2	28		1498.82	750.39	1499.83	750.42	500.61
SP20	Ac-F\$8AYWEAc3cL\$AAChg-NH2	29		1524.84	763.48	1525.85	763.43	509.29
SP21	Ac-F\$8AYWEAc3cL\$AACha-NH2	30		1538.85	770.44	1539.86	770.43	513.96
SP22	Ac-F\$8AYWEAc3cL\$AAib-NH2	31		1470.79	736.84	1471.8	736.4	491.27
SP23	Ac-LTF\$8AYWAQL\$AAibV-NH2	32		1771.01	885.81	1772.02	886.51	591.34
SP24	Ac-LTF\$8AYWAQL\$AAibV-NH2	33	iso2	1771.01	886.26	1772.02	886.51	591.34
SP25	Ac-LTF\$8AYWAQL\$SAibAA-NH2	34		1758.97	879.89	1759.98	880.49	587.33
SP26	Ac-LTF\$8AYWAQL\$SAibAA-NH2	35	iso2	1758.97	880.34	1759.98	880.49	587.33
SP27	Ac-HLTF\$8HHWHQL\$RRDCha-NH2	36		2056.15	1028.86	2057.16	1029.08	686.39
SP28	Ac-DLTF\$8HHWHQL\$RRRLV-NH2	37		2190.23	731.15	2191.24	1096.12	731.08
SP29	Ac-HHTF\$8HHWHQL\$AAML-NH2	38		2098.08	700.43	2099.09	1050.05	700.37
SP30	Ac-F\$8HHWHQL\$RRDCha-NH2	39		1917.06	959.96	1918.07	959.54	640.03
SP31	Ac-F\$8HHWHQL\$SHRFV-NH2	40		1876.02	938.65	1877.03	939.02	626.35
SP32	Ac-HLTF\$8HHWHQL\$AAhLA-NH2	41		2028.12	677.2	2029.13	1015.07	677.05
SP33	Ac-DLTF\$8HHWHQL\$RRChgl-NH2	42		2230.26	1115.89	2231.27	1116.14	744.43
SP34	Ac-DLTF\$8HHWHQL\$RRChgl-NH2	43	iso2	2230.26	1115.96	2231.27	1116.14	744.43
SP35	Ac-HHTF\$8HHWHQL\$AAChav-NH2	44		2106.14	1053.95	2107.15	1054.08	703.05
SP36	Ac-F\$8HHWHQL\$RRDA-NH2	45		1834.99	918.3	1836	918.5	612.67
SP37	Ac-F\$8HHWHQL\$SHRAibG-NH2	46		1771.95	886.77	1772.96	886.98	591.66
SP38	Ac-F\$8AYWAQL\$SHHNleL-NH2	47		1730.97	866.57	1731.98	866.49	578
SP39	Ac-F\$8AYWAL\$SHQANle-NH2	48		1638.89	820.54	1639.9	820.45	547.3
SP40	Ac-F\$8AYWVQL\$SQHChgl-NH2	49		1776.01	889.44	1777.02	889.01	593.01
SP41	Ac-F\$8AYWTAL\$SQNlev-NH2	50		1671.94	836.97	1672.95	836.98	558.32
SP42	Ac-F\$8AYWYQL\$SHAibAa-NH2	51		1686.89	844.52	1687.9	844.45	563.3
SP43	Ac-LTF\$8AYWAQL\$SHHLA-NH2	52		1903.05	952.27	1904.06	952.53	635.36
SP44	Ac-LTF\$8AYWAQL\$SHHLA-NH2	53	iso2	1903.05	952.27	1904.06	952.53	635.36
SP45	Ac-LTF\$8AYWAQL\$SHQNlev-NH2	54		1922.08	962.48	1923.09	962.05	641.7
SP46	Ac-LTF\$8AYWAQL\$SHQNlev-NH2	55	iso2	1922.08	962.4	1923.09	962.05	641.7
SP47	Ac-LTF\$8AYWAQL\$SQMI-NH2	56		1945.05	973.95	1946.06	973.53	649.36
SP48	Ac-LTF\$8AYWAQL\$SQMI-NH2	57	iso2	1945.05	973.88	1946.06	973.53	649.36
SP49	Ac-LTF\$8AYWAQL\$SHAibhLV-NH2	58		1893.09	948.31	1894.1	947.55	632.04
SP50	Ac-LTF\$8AYWAQL\$AHFA-NH2	59		1871.01	937.4	1872.02	936.51	624.68
SP51	Ac-HLTF\$8HHWHQL\$AANlel-NH2	60		2056.15	1028.79	2057.16	1029.08	686.39
SP52	Ac-DLTF\$8HHWHQL\$RRRLA-NH2	61		2162.2	721.82	2163.21	1082.11	721.74
SP53	Ac-HHTF\$8HHWHQL\$AAMv-NH2	62		2084.07	1042.92	2085.08	1043.04	695.7
SP54	Ac-F\$8HHWHQL\$RRDA-NH2	63		1834.99	612.74	1836	918.5	612.67
SP55	Ac-F\$8HHWHQL\$SHRFCha-NH2	64		1930.06	966.47	1931.07	966.04	644.36
SP56	Ac-F\$8AYWEAL\$AA-NHAm	65		1443.82	1445.71	1444.83	722.92	482.28
SP57	Ac-F\$8AYWEAL\$AA-NHiAm	66		1443.82	723.13	1444.83	722.92	482.28
SP58	Ac-F\$8AYWEAL\$AA-NHnPr3Ph	67		1491.82	747.3	1492.83	746.92	498.28
SP59	Ac-F\$8AYWEAL\$AA-NHnBu33Me	68		1457.83	1458.94	1458.84	729.92	486.95
SP60	Ac-F\$8AYWEAL\$AA-NHnPr	69		1415.79	709.28	1416.8	708.9	472.94
SP61	Ac-F\$8AYWEAL\$AA-NHnEt2Ch	70		1483.85	1485.77	1484.86	742.93	495.62
SP62	Ac-F\$8AYWEAL\$AA-NHnEt2Cp	71		1469.83	1470.78	1470.84	735.92	490.95
SP63	Ac-F\$8AYWEAL\$AA-NHHex	72		1457.83	730.19	1458.84	729.92	486.95
SP64	Ac-LTF\$8AYWAQL\$AAIA-NH2	73		1771.01	885.81	1772.02	886.51	591.34
SP65	Ac-LTF\$8AYWAQL\$AAIA-NH2	74	iso2	1771.01	866.8	1772.02	886.51	591.34
SP66	Ac-LTF\$8AYWAQL\$AAIA-NH2	75		1731.94	867.08	1732.95	866.98	578.32
SP67	Ac-LTF\$8AYWAAL\$AAIA-NH2	76	iso2	1731.94	867.28	1732.95	866.98	578.32
SP68	Ac-LTF\$8AYWAQL\$AANleA-NH2	77		1771.01	867.1	1772.02	886.51	591.34
SP69	Ac-LTF\$8AYWAQL\$AANleA-NH2	78	iso2	1771.01	886.89	1772.02	886.51	591.34
SP70	Ac-LTF\$8AYWAQL\$AAIA-NH2	79		1771.01	886.8	1772.02	886.51	591.34
SP71	Ac-LTF\$8AYWAQL\$AAIA-NH2	80	iso2	1771.01	887.09	1772.02	886.51	591.34
SP72	Ac-LTF\$8AYWAAL\$AAIA-NH2	81		1731.94	867.17	1732.95	866.98	578.32
SP73	Ac-LTF\$8AYWAAL\$AAIA-NH2	82	iso2	1731.94	867.37	1732.95	866.98	578.32

TABLE 1-continued

SP	Sequence	SEQ ID NO:	Isomer	Exact Mass	Found Mass	Calc (M + 1)/1	Calc (M + 2)/2	Calc (M + 3)/3
SP74	Ac-LTFsR8AYWAQL\$AANlea-NH2	83		1771.01	887.08	1772.02	886.51	591.34
SP75	Ac-LTFsR8AYWAQL\$AANlea-NH2	84	iso2	1771.01	887.08	1772.02	886.51	591.34
SP76	Ac-LTFsR8AYWAAL\$AAIv-NH2	85		1742.02	872.37	1743.03	872.02	581.68
SP77	Ac-LTFsR8AYWAAL\$AAIv-NH2	86	iso2	1742.02	872.74	1743.03	872.02	581.68
SP78	Ac-LTFsR8AYWAQL\$AAMv-NH2	87		1817	910.02	1818.01	909.51	606.67
SP79	Ac-LTFsR8AYWAAL\$AANlev-NH2	88		1742.02	872.37	1743.03	872.02	581.68
SP80	Ac-LTFsR8AYWAAL\$AANlev-NH2	89	iso2	1742.02	872.28	1743.03	872.02	581.68
SP81	Ac-LTFsR8AYWAQL\$AAII-NH2	90		1813.05	907.81	1814.06	907.53	605.36
SP82	Ac-LTFsR8AYWAQL\$AAII-NH2	91	iso2	1813.05	907.81	1814.06	907.53	605.36
SP83	Ac-LTFsR8AYWAAL\$AAMI-NH2	92		1773.99	887.37	1775	888	592.34
SP84	Ac-LTFsR8AYWAQL\$AANlel-NH2	93		1813.05	907.61	1814.06	907.53	605.36
SP85	Ac-LTFsR8AYWAQL\$AANlel-NH2	94	iso2	1813.05	907.71	1814.06	907.53	605.36
SP86	Ac-FsR8AYWEAL\$AAMA-NH2	95		1575.82	789.02	1576.83	788.92	526.28
SP87	Ac-FsR8AYWEAL\$AANleA-NH2	96		1557.86	780.14	1558.87	779.94	520.29
SP88	Ac-FsR8AYWEAL\$AAIa-NH2	97		1557.86	780.33	1558.87	779.94	520.29
SP89	Ac-FsR8AYWEAL\$AAMa-NH2	98		1575.82	789.3	1576.83	788.92	526.28
SP90	Ac-FsR8AYWEAL\$AANlea-NH2	99		1557.86	779.4	1558.87	779.94	520.29
SP91	Ac-FsR8AYWEAL\$AAIv-NH2	100		1585.89	794.29	1586.9	793.95	529.64
SP92	Ac-FsR8AYWEAL\$AAMv-NH2	101		1603.85	803.08	1604.86	802.93	535.62
SP93	Ac-FsR8AYWEAL\$AANlev-NH2	102		1585.89	793.46	1586.9	793.95	529.64
SP94	Ac-FsR8AYWEAL\$AAII-NH2	103		1599.91	800.49	1600.92	800.96	534.31
SP95	Ac-FsR8AYWEAL\$AAMI-NH2	104		1617.86	809.44	1618.87	809.94	540.29
SP96	Ac-FsR8AYWEAL\$AANlel-NH2	105		1599.91	801.7	1600.92	800.96	534.31
SP97	Ac-FsR8AYWEAL\$AANlel-NH2	106	iso2	1599.91	801.42	1600.92	800.96	534.31
SP98	Ac-LTFsR8AY6cIWAQL\$SAA-NH2	107		1707.88	855.72	1708.89	854.95	570.3
SP99	Ac-LTFsR8AY6cIWAQL\$SAA-NH2	108	iso2	1707.88	855.35	1708.89	854.95	570.3
SP100	Ac-WTFsR8FYWSQL\$AVAA-NH2	109		1922.01	962.21	1923.02	962.01	641.68
SP101	Ac-WTFsR8FYWSQL\$AVAA-NH2	110	iso2	1922.01	962.49	1923.02	962.01	641.68
SP102	Ac-WTFsR8VYWSQL\$AVA-NH2	111		1802.98	902.72	1803.99	902.5	602
SP103	Ac-WTFsR8VYWSQL\$AVA-NH2	112	iso2	1802.98	903	1803.99	902.5	602
SP104	Ac-WTFsR8FYWSQL\$SAAA-NH2	113		1909.98	956.47	1910.99	956	637.67
SP105	Ac-WTFsR8FYWSQL\$SAAA-NH2	114	iso2	1909.98	956.47	1910.99	956	637.67
SP106	Ac-WTFsR8VYWSQL\$AVAA-NH2	115		1945.05	974.15	1946.06	973.53	649.36
SP107	Ac-WTFsR8VYWSQL\$AVAA-NH2	116	iso2	1945.05	973.78	1946.06	973.53	649.36
SP108	Ac-LTFsR8AYWAQL\$AVG-NH2	117		1671.94	837.52	1672.95	836.98	558.32
SP109	Ac-LTFsR8AYWAQL\$AVG-NH2	118	iso2	1671.94	837.21	1672.95	836.98	558.32
SP110	Ac-LTFsR8AYWAQL\$AVQ-NH2	119		1742.98	872.74	1743.99	872.5	582
SP111	Ac-LTFsR8AYWAQL\$AVQ-NH2	120	iso2	1742.98	872.74	1743.99	872.5	582
SP112	Ac-LTFsR8AYWAQL\$SAa-NH2	121		1673.92	838.23	1674.93	837.97	558.98
SP113	Ac-LTFsR8AYWAQL\$SAa-NH2	122	iso2	1673.92	838.32	1674.93	837.97	558.98
SP114	Ac-LTFsR8AYWAQL\$SLA-NH2	123		1687.93	844.37	1688.94	844.97	563.65
SP115	Ac-LTFsR8AYWAQL\$SLA-NH2	124	iso2	1687.93	844.81	1688.94	844.97	563.65
SP116	Ac-LTFsR8AYWEQL\$SAS-NH2	125		1826	905.27	1827.01	914.01	609.67
SP117	Ac-LTFsR8AYWAQL\$SLA-NH2	126		1715.97	858.48	1716.98	858.99	573
SP118	Ac-LTFsR8AYWAQL\$SLA-NH2	127	iso2	1715.97	858.87	1716.98	858.99	573
SP119	Ac-LTFsR8AYWAQL\$SSWA-NH2	128		1788.96	895.21	1789.97	895.49	597.33
SP120	Ac-LTFsR8AYWAQL\$SSWA-NH2	129	iso2	1788.96	895.28	1789.97	895.49	597.33
SP121	Ac-LTFsR8AYWAQL\$SVS-NH2	130		1717.94	859.84	1718.95	859.98	573.65
SP122	Ac-LTFsR8AYWAQL\$SAS-NH2	131		1689.91	845.85	1690.92	845.96	564.31
SP123	Ac-LTFsR8AYWAQL\$SVG-NH2	132		1687.93	844.81	1688.94	844.97	563.65
SP124	Ac-ETFsR8VYWAQL\$SAA-NH2	133		1717.91	859.76	1718.92	859.96	573.64
SP125	Ac-ETFsR8VYWAQL\$SAA-NH2	134		1717.91	859.84	1718.92	859.96	573.64
SP126	Ac-ETFsR8VYWAQL\$SVA-NH2	135		1745.94	873.82	1746.95	873.98	582.99
SP127	Ac-ETFsR8VYWAQL\$SLA-NH2	136		1759.96	880.85	1760.97	880.99	587.66
SP128	Ac-ETFsR8VYWAQL\$SSWA-NH2	137		1832.95	917.34	1833.96	917.48	611.99
SP129	Ac-ETFsR8KYWAQL\$SSWA-NH2	138		1861.98	931.92	1862.99	932	621.67
SP130	Ac-ETFsR8VYWAQL\$SVS-NH2	139		1761.93	881.89	1762.94	881.97	588.32
SP131	Ac-ETFsR8VYWAQL\$SAS-NH2	140		1733.9	867.83	1734.91	867.96	578.97
SP132	Ac-ETFsR8VYWAQL\$SVG-NH2	141		1731.92	866.87	1732.93	866.97	578.31
SP133	Ac-LTFsR8VYWAQL\$SSa-NH2	142		1717.94	859.47	1718.95	859.98	573.65
SP134	Ac-ETFsR8VYWAQL\$SSa-NH2	143		1733.9	867.83	1734.91	867.96	578.97
SP135	Ac-LTFsR8VYWAQL\$SSNa-NH2	144		1744.96	873.38	1745.97	873.49	582.66
SP136	Ac-ETFsR8VYWAQL\$SSNa-NH2	145		1760.91	881.3	1761.92	881.46	587.98
SP137	Ac-LTFsR8VYWAQL\$SAa-NH2	146		1701.95	851.84	1702.96	851.98	568.32
SP138	Ac-LTFsR8VYWAQL\$SVA-NH2	147		1729.98	865.53	1730.99	866	577.67
SP139	Ac-LTFsR8VYWAQL\$SVA-NH2	148	iso2	1729.98	865.9	1730.99	866	577.67
SP140	Ac-LTFsR8VYWAQL\$SSWA-NH2	149		1816.99	909.42	1818	909.5	606.67
SP141	Ac-LTFsR8VYWAQL\$SVS-NH2	150		1745.98	873.9	1746.99	874	583
SP142	Ac-LTFsR8VYWAQL\$SVS-NH2	151	iso2	1745.98	873.9	1746.99	874	583
SP143	Ac-LTFsR8VYWAQL\$SAS-NH2	152		1717.94	859.84	1718.95	859.98	573.65
SP144	Ac-LTFsR8VYWAQL\$SAS-NH2	153	iso2	1717.94	859.91	1718.95	859.98	573.65
SP145	Ac-LTFsR8VYWAQL\$SVG-NH2	154		1715.97	858.87	1716.98	858.99	573
SP146	Ac-LTFsR8VYWAQL\$SVG-NH2	155	iso2	1715.97	858.87	1716.98	858.99	573
SP147	Ac-LTFsR8EYWAQCh\$SAA-NH2	156		1771.96	886.85	1772.97	886.99	591.66
SP148	Ac-LTFsR8EYWAQCh\$SAA-NH2	157	iso2	1771.96	886.85	1772.97	886.99	591.66
SP149	Ac-LTFsR8EYWAQCh\$SAA-NH2	158		1743.92	872.86	1744.93	872.97	582.31

TABLE 1-continued

SP	Sequence	SEQ ID NO:	Isomer	Exact Mass	Found Mass	Calc (M + 1)/1	Calc (M + 2)/2	Calc (M + 3)/3
SP150	Ac-LTF\$8EYWAQCpg\$SAA-NH2	159	iso2	1743.92	872.86	1744.93	872.97	582.31
SP151	Ac-LTF\$8EYWAQF\$SAA-NH2	160		1765.91	883.44	1766.92	883.96	589.64
SP152	Ac-LTF\$8EYWAQF\$SAA-NH2	161	iso2	1765.91	883.89	1766.92	883.96	589.64
SP153	Ac-LTF\$8EYWAQCba\$SAA-NH2	162		1743.92	872.42	1744.93	872.97	582.31
SP154	Ac-LTF\$8EYWAQCba\$SAA-NH2	163	iso2	1743.92	873.39	1744.93	872.97	582.31
SP155	Ac-LTF3C1\$8EYWAQL\$SAA-NH2	164		1765.89	883.89	1766.9	883.95	589.64
SP156	Ac-LTF3C1\$8EYWAQL\$SAA-NH2	165	iso2	1765.89	883.96	1766.9	883.95	589.64
SP157	Ac-LTF34F2\$8EYWAQL\$SAA-NH2	166		1767.91	884.48	1768.92	884.96	590.31
SP158	Ac-LTF34F2\$8EYWAQL\$SAA-NH2	167	iso2	1767.91	884.48	1768.92	884.96	590.31
SP159	Ac-LTF34F2\$8EYWAQhL\$SAA-NH2	168		1781.92	891.44	1782.93	891.97	594.98
SP160	Ac-LTF34F2\$8EYWAQhL\$SAA-NH2	169	iso2	1781.92	891.88	1782.93	891.97	594.98
SP161	Ac-ETF\$8EYWAQL\$SAA-NH2	170		1747.88	874.34	1748.89	874.95	583.63
SP162	Ac-LTF\$8AYWVQL\$SAA-NH2	171		1701.95	851.4	1702.96	851.98	568.32
SP163	Ac-LTF\$8AHWAQL\$SAA-NH2	172		1647.91	824.83	1648.92	824.96	550.31
SP164	Ac-LTF\$8AEWAQL\$SAA-NH2	173		1639.9	820.39	1640.91	820.96	547.64
SP165	Ac-LTF\$8ASWAQL\$SAA-NH2	174		1597.89	799.38	1598.9	799.95	533.64
SP166	Ac-LTF\$8AEWAQL\$SAA-NH2	175	iso2	1639.9	820.39	1640.91	820.96	547.64
SP167	Ac-LTF\$8AEWAQL\$SAA-NH2	176	iso2	1597.89	800.31	1598.9	799.95	533.64
SP168	Ac-LTF\$8AF4coohWAQL\$SAA-NH2	177		1701.91	851.4	1702.92	851.96	568.31
SP169	Ac-LTF\$8AF4coohWAQL\$SAA-NH2	178	iso2	1701.91	851.4	1702.92	851.96	568.31
SP170	Ac-LTF\$8AHWAQL\$AAIa-NH2	179		1745	874.13	1746.01	873.51	582.67
SP171	Ac-ITF\$8FYWAQL\$AAIa-NH2	180		1847.04	923.92	1848.05	924.53	616.69
SP172	Ac-ITF\$8EHWAQL\$AAIa-NH2	181		1803.01	903.17	1804.02	902.51	602.01
SP173	Ac-ITF\$8EHWAQL\$AAIa-NH2	182	iso2	1803.01	903.17	1804.02	902.51	602.01
SP174	Ac-ETF\$8EHWAQL\$AAIa-NH2	183		1818.97	910.76	1819.98	910.49	607.33
SP175	Ac-ETF\$8EHWAQL\$AAIa-NH2	184	iso2	1818.97	910.85	1819.98	910.49	607.33
SP176	Ac-LTF\$8AHWVQL\$AAIa-NH2	185		1773.03	888.09	1774.04	887.52	592.02
SP177	Ac-ITF\$8FYWVQL\$AAIa-NH2	186		1875.07	939.16	1876.08	938.54	626.03
SP178	Ac-ITF\$8EYWVQL\$AAIa-NH2	187		1857.04	929.83	1858.05	929.53	620.02
SP179	Ac-ITF\$8EHWVQL\$AAIa-NH2	188		1831.04	916.86	1832.05	916.53	611.35
SP180	Ac-LTF\$8AEWAQL\$AAIa-NH2	189		1736.99	869.87	1738	869.5	580
SP181	Ac-LTF\$8AF4coohWAQL\$AAIa-NH2	190		1799	900.17	1800.01	900.51	600.67
SP182	Ac-LTF\$8AF4coohWAQL\$AAIa-NH2	191	iso2	1799	900.24	1800.01	900.51	600.67
SP183	Ac-LTF\$8AHWAQL\$AHFA-NH2	192		1845.01	923.89	1846.02	923.51	616.01
SP184	Ac-ITF\$8FYWAQL\$AHFA-NH2	193		1947.05	975.05	1948.06	974.53	650.02
SP185	Ac-ITF\$8FYWAQL\$AHFA-NH2	194	iso2	1947.05	976.07	1948.06	974.53	650.02
SP186	Ac-ITF\$8FHWAQL\$AEFA-NH2	195		1913.02	958.12	1914.03	957.52	638.68
SP187	Ac-ITF\$8FHWAQL\$AEFA-NH2	196	iso2	1913.02	957.86	1914.03	957.52	638.68
SP188	Ac-ITF\$8EHWAQL\$AHFA-NH2	197		1903.01	952.94	1904.02	952.51	635.34
SP189	Ac-ITF\$8EHWAQL\$AHFA-NH2	198	iso2	1903.01	953.87	1904.02	952.51	635.34
SP190	Ac-LTF\$8AHWVQL\$AHFA-NH2	199		1873.04	937.86	1874.05	937.53	625.35
SP191	Ac-ITF\$8FYWVQL\$AHFA-NH2	200		1975.08	988.83	1976.09	988.55	659.37
SP192	Ac-ITF\$8EYWVQL\$AHFA-NH2	201		1957.05	979.35	1958.06	979.53	653.36
SP193	Ac-ITF\$8EHWVQL\$AHFA-NH2	202		1931.05	967	1932.06	966.53	644.69
SP194	Ac-ITF\$8EHWVQL\$AHFA-NH2	203	iso2	1931.05	967.93	1932.06	966.53	644.69
SP195	Ac-ETF\$8EYWAAL\$SAA-NH2	204		1690.86	845.85	1691.87	846.44	564.63
SP196	Ac-LTF\$8AYWVAL\$SAA-NH2	205		1644.93	824.08	1645.94	823.47	549.32
SP197	Ac-LTF\$8AHWAAL\$SAA-NH2	206		1590.89	796.88	1591.9	796.45	531.3
SP198	Ac-LTF\$8AEWAAL\$SAA-NH2	207		1582.88	791.9	1583.89	792.45	528.63
SP199	Ac-LTF\$8AEWAAL\$SAA-NH2	208	iso2	1582.88	791.9	1583.89	792.45	528.63
SP200	Ac-LTF\$8ASWAAL\$SAA-NH2	209		1540.87	770.74	1541.88	771.44	514.63
SP201	Ac-LTF\$8ASWAAL\$SAA-NH2	210	iso2	1540.87	770.88	1541.88	771.44	514.63
SP202	Ac-LTF\$8AYWAAL\$AAIa-NH2	211		1713.99	857.39	1715	858	572.34
SP203	Ac-LTF\$8AYWAAL\$AAIa-NH2	212	iso2	1713.99	857.84	1715	858	572.34
SP204	Ac-LTF\$8AYWAAL\$AHFA-NH2	213		1813.99	907.86	1815	908	605.67
SP205	Ac-LTF\$8EHWAQL\$AHIa-NH2	214		1869.03	936.1	1870.04	935.52	624.02
SP206	Ac-LTF\$8EHWAQL\$AHIa-NH2	215	iso2	1869.03	937.03	1870.04	935.52	624.02
SP207	Ac-LTF\$8AHWAQL\$AHIa-NH2	216		1811.03	906.87	1812.04	906.52	604.68
SP208	Ac-LTF\$8EYWAQL\$AHIa-NH2	217		1895.04	949.15	1896.05	948.53	632.69
SP209	Ac-LTF\$8AYWAQL\$AAFa-NH2	218		1804.99	903.2	1806	903.5	602.67
SP210	Ac-LTF\$8AYWAQL\$AAFa-NH2	219	iso2	1804.99	903.28	1806	903.5	602.67
SP211	Ac-LTF\$8AYWAQL\$AAWa-NH2	220		1844	922.81	1845.01	923.01	615.67
SP212	Ac-LTF\$8AYWAQL\$AAVa-NH2	221		1756.99	878.86	1758	879.5	586.67
SP213	Ac-LTF\$8AYWAQL\$AAVa-NH2	222	iso2	1756.99	879.3	1758	879.5	586.67
SP214	Ac-LTF\$8AYWAQL\$AAIa-NH2	223		1771.01	886.26	1772.02	886.51	591.34
SP215	Ac-LTF\$8AYWAQL\$AAIa-NH2	224	iso2	1771.01	886.33	1772.02	886.51	591.34
SP216	Ac-LTF\$8EYWAQL\$AAIa-NH2	225		1829.01	914.89	1830.02	915.51	610.68
SP217	Ac-LTF\$8EYWAQL\$AAIa-NH2	226	iso2	1829.01	915.34	1830.02	915.51	610.68
SP218	Ac-LTF\$8EYWAQL\$AAFa-NH2	227		1863	932.87	1864.01	932.51	622.01
SP219	Ac-LTF\$8EYWAQL\$AAFa-NH2	228	iso2	1863	932.87	1864.01	932.51	622.01
SP220	Ac-LTF\$8EYWAQL\$AAVa-NH2	229		1815	908.23	1816.01	908.51	606.01
SP221	Ac-LTF\$8EYWAQL\$AAVa-NH2	230	iso2	1815	908.31	1816.01	908.51	606.01
SP222	Ac-LTF\$8EHWAQL\$AAIa-NH2	231		1803.01	903.17	1804.02	902.51	602.01
SP223	Ac-LTF\$8EHWAQL\$AAIa-NH2	232	iso2	1803.01	902.8	1804.02	902.51	602.01
SP224	Ac-LTF\$8EHWAQL\$AAWa-NH2	233		1876	939.34	1877.01	939.01	626.34
SP225	Ac-LTF\$8EHWAQL\$AAWa-NH2	234	iso2	1876	939.62	1877.01	939.01	626.34

TABLE 1-continued

SP	Sequence	SEQ ID NO:	Isomer	Exact Mass	Found Mass	Calc (M + 1)/1	Calc (M + 2)/2	Calc (M + 3)/3
SP226	Ac-LTFs8EHWAQL\$AALa-NH2	235		1803.01	902.8	1804.02	902.51	602.01
SP227	Ac-LTFs8EHWAQL\$AALa-NH2	236	iso2	1803.01	902.9	1804.02	902.51	602.01
SP228	Ac-ETFs8EHWVQL\$AALa-NH2	237		1847	924.82	1848.01	924.51	616.67
SP229	Ac-LTFs8AYWAQL\$AAAa-NH2	238		1728.96	865.89	1729.97	865.49	577.33
SP230	Ac-LTFs8AYWAQL\$AAAa-NH2	239	iso2	1728.96	865.89	1729.97	865.49	577.33
SP231	Ac-LTFs8AYWAQL\$AAAib-NH2	240		1742.98	872.83	1743.99	872.5	582
SP232	Ac-LTFs8AYWAQL\$AAAib-NH2	241	iso2	1742.98	872.92	1743.99	872.5	582
SP233	Ac-LTFs8AYWAQL\$AAAAa-NH2	242		1800	901.42	1801.01	901.01	601.01
SP234	Ac-LTFs5AYWAQL\$8AAIa-NH2	243		1771.01	887.17	1772.02	886.51	591.34
SP235	Ac-LTFs5AYWAQL\$8SAA-NH2	244		1673.92	838.33	1674.93	837.97	558.98
SP236	Ac-LTFs8AYWAQCba\$AANleA-NH2	245		1783.01	892.64	1784.02	892.51	595.34
SP237	Ac-ETFs8AYWAQCba\$AANleA-NH2	246		1798.97	900.59	1799.98	900.49	600.66
SP238	Ac-LTFs8EYWAQCba\$AANleA-NH2	247		1841.01	922.05	1842.02	921.51	614.68
SP239	Ac-LTFs8AYWAQCba\$AWNleA-NH2	248		1898.05	950.46	1899.06	950.03	633.69
SP240	Ac-ETFs8AYWAQCba\$AWNleA-NH2	249		1914.01	958.11	1915.02	958.01	639.01
SP241	Ac-LTFs8EYWAQCba\$AWNleA-NH2	250		1956.06	950.62	1957.07	979.04	653.03
SP242	Ac-LTFs8EYWAQCba\$SAFA-NH2	251		1890.99	946.55	1892	946.5	631.34
SP243	Ac-LTF34F2S8EYWAQCba\$SANleA-NH2	252		1892.99	947.57	1894	947.5	632
SP244	Ac-LTFs8EF4coohWAQCba\$SANleA-NH2	253		1885	943.59	1886.01	943.51	629.34
SP245	Ac-LTFs8EYWSQCba\$SANleA-NH2	254		1873	937.58	1874.01	937.51	625.34
SP246	Ac-LTFs8EYWWQCba\$SANleA-NH2	255		1972.05	987.61	1973.06	987.03	658.36
SP247	Ac-LTFs8EYWAQCba\$AAIa-NH2	256		1841.01	922.05	1842.02	921.51	614.68
SP248	Ac-LTF34F2S8EYWAQCba\$AAIa-NH2	257		1876.99	939.99	1878	939.5	626.67
SP249	Ac-LTFs8EF4coohWAQCba\$AAIa-NH2	258		1869.01	935.64	1870.02	935.51	624.01
SP250	Pam-ETFs8EYWAQCba\$SAA-NH2	259		1956.1	979.57	1957.11	979.06	653.04
SP251	Ac-LTFs8EFWAQCba\$SAA-NH2	260		1741.94	872.11	1742.95	871.98	581.65
SP252	Ac-LTA8EYWAQCba\$SAA-NH2	261		1667.89	835.4	1668.9	834.95	556.97
SP253	Ac-LTFs8EYAAQCba\$SAA-NH2	262		1628.88	815.61	1629.89	815.45	543.97
SP254	Ac-LTFs8EY2NaIQCba\$SAA-NH2	263		1754.93	879.04	1755.94	878.47	585.98
SP255	Ac-LTFs8AYWAQCba\$SAA-NH2	264		1685.92	844.71	1686.93	843.97	562.98
SP256	Ac-LTFs8EYWAQCba\$SAF-NH2	265		1819.96	911.41	1820.97	910.99	607.66
SP257	Ac-LTFs8EYWAQCba\$SAFA-NH2	266		1890.99	947.41	1892	946.5	631.34
SP258	Ac-LTFs8AYWAQCba\$SAF-NH2	267		1761.95	882.73	1762.96	881.98	588.32
SP259	Ac-LTF34F2S8AYWAQCba\$SAF-NH2	268		1797.93	900.87	1798.94	899.97	600.32
SP260	Ac-LTFs8AF4coohWAQCba\$SAF-NH2	269		1789.94	896.43	1790.95	895.98	597.65
SP261	Ac-LTFs8EY6elWAQCba\$SAF-NH2	270		1853.92	929.27	1854.93	927.97	618.98
SP262	Ac-LTFs8AYWSQCba\$SAF-NH2	271		1777.94	890.87	1778.95	889.98	593.65
SP263	Ac-LTFs8AYWWQCba\$SAF-NH2	272		1876.99	939.91	1878	939.5	626.67
SP264	Ac-LTFs8AYWAQCba\$AAIa-NH2	273		1783.01	893.19	1784.02	892.51	595.34
SP265	Ac-LTF34F2S8AYWAQCba\$AAIa-NH2	274		1818.99	911.23	1820	910.5	607.34
SP266	Ac-LTFs8AY6elWAQCba\$AAIa-NH2	275		1816.97	909.84	1817.98	909.49	606.66
SP267	Ac-LTFs8AF4coohWAQCba\$AAIa-NH2	276		1811	906.88	1812.01	906.51	604.67
SP268	Ac-LTFs8EYWAQCba\$AAFa-NH2	277		1875	938.6	1876.01	938.51	626.01
SP269	Ac-LTFs8EYWAQCba\$AAFa-NH2	278	iso2	1875	938.6	1876.01	938.51	626.01
SP270	Ac-ETFs8AYWAQCba\$AWNleA-NH2	279		1914.01	958.42	1915.02	958.01	639.01
SP271	Ac-LTFs8EYWAQCba\$AWNleA-NH2	280		1956.06	979.42	1957.07	979.04	653.03
SP272	Ac-ETFs8EYWAQCba\$AWNleA-NH2	281		1972.01	987.06	1973.02	987.01	658.34
SP273	Ac-ETFs8EYWAQCba\$AWNleA-NH2	282	iso2	1972.01	987.06	1973.02	987.01	658.34
SP274	Ac-LTFs8AYWAQCba\$SAFa-NH2	283		1832.99	917.89	1834	917.5	612
SP275	Ac-LTFs8AYWAQCba\$SAFa-NH2	284	iso2	1832.99	918.07	1834	917.5	612
SP276	Ac-ETFs8AYWAQCba\$AWNleA-NH2	285		1902.01	952.22	1903.02	952.01	635.01
SP277	Ac-LTFs8EYWAQL\$AWNleA-NH2	286		1944.06	973.5	1945.07	973.04	649.03
SP278	Ac-ETFs8EYWAQL\$AWNleA-NH2	287		1960.01	981.46	1961.02	981.01	654.34
SP279	Dmaac-LTFs8EYWAQhL\$SAA-NH2	288		1788.98	896.06	1789.99	895.5	597.33
SP280	Hexac-LTFs8EYWAQhL\$SAA-NH2	289		1802	902.9	1803.01	902.01	601.67
SP281	Napac-LTFs8EYWAQhL\$SAA-NH2	290		1871.99	937.58	1873	937	625
SP282	Decac-LTFs8EYWAQhL\$SAA-NH2	291		1858.06	930.55	1859.07	930.04	620.36
SP283	Admac-LTFs8EYWAQhL\$SAA-NH2	292		1866.03	934.07	1867.04	934.02	623.02
SP284	Tmac-LTFs8EYWAQhL\$SAA-NH2	293		1787.99	895.41	1789	895	597
SP285	Pam-LTFs8EYWAQhL\$SAA-NH2	294		1942.16	972.08	1943.17	972.09	648.39
SP286	Ac-LTFs8AYWAQCba\$AANleA-NH2	295	iso2	1783.01	892.64	1784.02	892.51	595.34
SP287	Ac-LTF34F2S8EYWAQCba\$AAIa-NH2	296	iso2	1876.99	939.62	1878	939.5	626.67
SP288	Ac-LTF34F2S8EYWAQCba\$SAA-NH2	297		1779.91	892.07	1780.92	890.96	594.31
SP289	Ac-LTF34F2S8EYWAQCba\$SAA-NH2	298	iso2	1779.91	891.61	1780.92	890.96	594.31
SP290	Ac-LTFs8EF4coohWAQCba\$SAA-NH2	299		1771.92	887.54	1772.93	886.97	591.65
SP291	Ac-LTFs8EF4coohWAQCba\$SAA-NH2	300	iso2	1771.92	887.63	1772.93	886.97	591.65
SP292	Ac-LTFs8EYWSQCba\$SAA-NH2	301		1759.92	881.9	1760.93	880.97	587.65
SP293	Ac-LTFs8EYWSQCba\$SAA-NH2	302	iso2	1759.92	881.9	1760.93	880.97	587.65
SP294	Ac-LTFs8EYWAQhL\$SAA-NH2	303		1745.94	875.05	1746.95	873.98	582.99
SP295	Ac-LTFs8AYWAQhL\$SAF-NH2	304		1763.97	884.02	1764.98	882.99	589
SP296	Ac-LTFs8AYWAQhL\$SAF-NH2	305	iso2	1763.97	883.56	1764.98	882.99	589
SP297	Ac-LTF34F2S8AYWAQhL\$SAA-NH2	306		1723.92	863.67	1724.93	862.97	575.65
SP298	Ac-LTF34F2S8AYWAQhL\$SAA-NH2	307	iso2	1723.92	864.04	1724.93	862.97	575.65
SP299	Ac-LTFs8AF4coohWAQhL\$SAA-NH2	308		1715.93	859.44	1716.94	858.97	572.98
SP300	Ac-LTFs8AF4coohWAQhL\$SAA-NH2	309	iso2	1715.93	859.6	1716.94	858.97	572.98
SP301	Ac-LTFs8AYWSQhL\$SAA-NH2	310		1703.93	853.96	1704.94	852.97	568.98

TABLE 1-continued

SP	Sequence	SEQ ID NO:	Isomer	Exact Mass	Found Mass	Calc (M + 1)/1	Calc (M + 2)/2	Calc (M + 3)/3
SP302	Ac-LTFsR8AYWSQhL\$SAA-NH2	311	iso2	1703.93	853.59	1704.94	852.97	568.98
SP303	Ac-LTFsR8EYWAQL\$SAA-NH2	312		1829.01	915.45	1830.02	915.51	610.68
SP304	Ac-LTF34F2S8AYWAQL\$SAA-NH2	313		1806.99	904.58	1808	904.5	603.34
SP305	Ac-LTFsR8AF4coohWAQL\$SAA-NH2	314		1799	901.6	1800.01	900.51	600.67
SP306	Ac-LTFsR8AYWSQL\$SAA-NH2	315		1787	894.75	1788.01	894.51	596.67
SP307	Ac-LTF34F2S8AYWAQL\$SAA-NH2	316		1821	911.79	1822.01	911.51	608.01
SP308	Ac-LTF34F2S8AYWAQhL\$SAA-NH2	317	iso2	1821	912.61	1822.01	911.51	608.01
SP309	Ac-LTFsR8AF4coohWAQhL\$SAA-NH2	318		1813.02	907.95	1814.03	907.52	605.35
SP310	Ac-LTFsR8AF4coohWAQhL\$SAA-NH2	319	iso2	1813.02	908.54	1814.03	907.52	605.35
SP311	Ac-LTFsR8AYWSQhL\$SAA-NH2	320		1801.02	901.84	1802.03	901.52	601.35
SP312	Ac-LTFsR8AYWSQhL\$SAA-NH2	321	iso2	1801.02	902.62	1802.03	901.52	601.35
SP313	Ac-LTFsR8AYWAQhL\$SAA-NH2	322		1814.01	908.63	1815.02	908.01	605.68
SP314	Ac-LTFsR8AYWAQhL\$SAA-NH2	323	iso2	1814.01	908.34	1815.02	908.01	605.68
SP315	Ac-LTFsR8AYWAQL\$SAA-NH2	324		1871.04	936.94	1872.05	936.53	624.69
SP316	Ac-LTFsR8AYWAQL\$SAA-NH2	325	iso2	1942.07	972.5	1943.08	972.04	648.37
SP317	Ac-LTFsR8AYWAQL\$SAA-NH2	326	iso1	1942.07	972.5	1943.08	972.04	648.37
SP318	Ac-LTFsR8EYWAQhL\$SAA-NH2	327		1843.03	922.54	1844.04	922.52	615.35
SP319	Ac-AATFsR8AYWAQL\$SAA-NH2	328		1800	901.39	1801.01	901.01	601.01
SP320	Ac-LTFsR8AYWAQL\$SAA-NH2	329		1842.04	922.45	1843.05	922.03	615.02
SP321	Ac-ALTFsR8AYWAQL\$SAA-NH2	330		1913.08	957.94	1914.09	957.55	638.7
SP322	Ac-LTFsR8EYWAQCb\$SAA-NH2	331		1854.04	928.43	1855.05	928.03	619.02
SP323	Ac-LTFsR8AYWAQhL\$SAA-NH2	332		1856.06	929.4	1857.07	929.04	619.69
SP324	Ac-LTFsR8EYWAQCb\$SAA-NH2	333		1814.96	909.37	1815.97	908.49	605.99
SP325	Ac-LTFsR8EYWAQCb\$SAA-NH2	334	iso2	1814.96	909.37	1815.97	908.49	605.99
SP326	Ac-LTFsR8EYWAQCb\$SAA-NH2	335		1886	944.61	1887.01	944.01	629.67
SP327	Ac-LTFsR8EYWAQCb\$SAA-NH2	336	iso2	1886	944.61	1887.01	944.01	629.67
SP328	Ac-ALTFsR8EYWAQCb\$SAA-NH2	337		1814.96	909.09	1815.97	908.49	605.99
SP329	Ac-ALTFsR8EYWAQCb\$SAA-NH2	338		1886	944.61	1887.01	944.01	629.67
SP330	Ac-ALTFsR8EYWAQCb\$SAA-NH2	339	iso2	1814.96	909.09	1815.97	908.49	605.99
SP331	Ac-LTFsR8EYWAQCb\$SAA-NH2	340	iso2	1929.04	966.08	1930.05	965.53	644.02
SP332	Ac-LTFsR8EY6clWAQCb\$SAA-NH2	341		1777.89	890.78	1778.9	889.95	593.64
SP333	Ac-LTFsR8EF4cooh6clWAQCb\$SAA-NH2	342		1918.96	961.27	1919.97	960.49	640.66
SP334	Ac-LTFsR8EF4cooh6clWAQCb\$SAA-NH2	343	iso2	1918.96	961.27	1919.97	960.49	640.66
SP335	Ac-LTFsR8EF4cooh6clWAQCb\$SAA-NH2	344		1902.97	953.03	1903.98	952.49	635.33
SP336	Ac-LTFsR8EF4cooh6clWAQCb\$SAA-NH2	345	iso2	1902.97	953.13	1903.98	952.49	635.33
SP337	Ac-LTFsR8AY6clWAQL\$SAA-NH2	346		1905	954.61	1906.01	953.51	636.01
SP338	Ac-LTFsR8AY6clWAQL\$SAA-NH2	347	iso2	1905	954.9	1906.01	953.51	636.01
SP339	Ac-FsR8AY6clWEAL\$SAA-NH2	348		1762.89	883.01	1763.9	882.45	588.64
SP340	Ac-ETFsR8EYWAQL\$SAA-NH2	349		1945	974.31	1946.01	973.51	649.34
SP341	Ac-ETFsR8EYWAQL\$SAA-NH2	350	iso2	1945	974.49	1946.01	973.51	649.34
SP342	Ac-LTFsR8EYWAQL\$SAA-NH2	351		2000.08	1001.6	2001.09	1001.05	667.7
SP343	Ac-LTFsR8EYWAQL\$SAA-NH2	352	iso2	2000.08	1001.6	2001.09	1001.05	667.7
SP344	Ac-LTFsR8AYWAQL\$SAA-NH2	353		1913.08	958.58	1914.09	957.55	638.7
SP345	Ac-LTFsR8AYWAQL\$SAA-NH2	354	iso2	1913.08	958.58	1914.09	957.55	638.7
SP346	Ac-LTFsR8EYWAQCb\$SAA-NH2	355		1941.04	972.55	1942.05	971.53	648.02
SP347	Ac-LTFsR8EYWAQCb\$SAA-NH2	356	iso2	1941.04	972.55	1942.05	971.53	648.02
SP348	Ac-LTFsR8EF4coohWAQCb\$SAA-NH2	357		1969.04	986.33	1970.05	985.53	657.35
SP349	Ac-LTFsR8EF4coohWAQCb\$SAA-NH2	358	iso2	1969.04	986.06	1970.05	985.53	657.35
SP350	Ac-LTFsR8EYWSQCb\$SAA-NH2	359		1957.04	980.04	1958.05	979.53	653.35
SP351	Ac-LTFsR8EYWSQCb\$SAA-NH2	360	iso2	1957.04	980.04	1958.05	979.53	653.35
SP352	Ac-LTFsR8EYWAQCb\$SAA-NH2	361		1814.96	909	1815.97	908.49	605.99
SP353	Ac-LTFsR8EYWAQCb\$SAA-NH2	362	iso2	1814.96	909	1815.97	908.49	605.99
SP354	Ac-ALTFsR8EYWAQCb\$SAA-NH2	363		1886	944.52	1887.01	944.01	629.67
SP355	Ac-ALTFsR8EYWAQCb\$SAA-NH2	364	iso2	1886	944.98	1887.01	944.01	629.67
SP356	Ac-ALTFsR8EYWAQCb\$SAA-NH2	365		1957.04	980.04	1958.05	979.53	653.35
SP357	Ac-ALTFsR8EYWAQCb\$SAA-NH2	366	iso2	1957.04	980.04	1958.05	979.53	653.35
SP358	Ac-AALTFsR8EYWAQCb\$SAA-NH2	367		2028.07	1016.1	2029.08	1015.04	677.03
SP359	Ac-AALTFsR8EYWAQCb\$SAA-NH2	368	iso2	2028.07	1015.57	2029.08	1015.04	677.03
SP360	Ac-RTFsR8EYWAQCb\$SAA-NH2	369		1786.94	895.03	1787.95	894.48	596.65
SP361	Ac-LRFsR8EYWAQCb\$SAA-NH2	370		1798.98	901.51	1799.99	900.5	600.67
SP362	Ac-LTFsR8EYWRQCb\$SAA-NH2	371		1828.99	916.4	1830	915.5	610.67
SP363	Ac-LTFsR8EYWARCb\$SAA-NH2	372		1771.97	887.63	1772.98	886.99	591.66
SP364	Ac-LTFsR8EYWAQCb\$SAA-NH2	373		1812.99	908.08	1814	907.5	605.34
SP365	Ac-LTFsR8EYWAQCb\$SAA-NH2	374		1828.99	916.12	1830	915.5	610.67
SP366	Ac-LTFsR8EYWAQCb\$SAA-NH2	375		1828.99	916.12	1830	915.5	610.67
SP367	5-FAM-BaLTFsR8EYWAQCb\$SAA-NH2	376		2131	1067.09	2132.01	1066.51	711.34
SP368	5-FAM-BaLTFsR8AYWAQL\$SAA-NH2	377		2158.08	1080.6	2159.09	1080.05	720.37
SP369	Ac-LAFsR8EYWAQL\$SAA-NH2	378		1799	901.05	1800.01	900.51	600.67
SP370	Ac-ATFsR8EYWAQL\$SAA-NH2	379		1786.97	895.03	1787.98	894.49	596.66
SP371	Ac-AAFsR8EYWAQL\$SAA-NH2	380		1756.96	880.05	1757.97	879.49	586.66
SP372	Ac-AAAFsR8EYWAQL\$SAA-NH2	381		1827.99	915.57	1829	915	610.34
SP373	Ac-AAAFsR8EYWAQL\$SAA-NH2	382		1899.03	951.09	1900.04	950.52	634.02
SP374	Ac-AATFsR8EYWAQL\$SAA-NH2	383		1858	930.92	1859.01	930.01	620.34
SP375	Ac-AALTFsR8EYWAQL\$SAA-NH2	384		1971.09	987.17	1972.1	986.55	658.04
SP376	Ac-AAALTFsR8EYWAQL\$SAA-NH2	385		2042.12	1023.15	2043.13	1022.07	681.71
SP377	Ac-LTFsR8EYWAQL\$SAA-NH2	386		1900.05	952.02	1901.06	951.03	634.36

TABLE 1-continued

SP	Sequence	SEQ ID NO:	Isomer	Exact Mass	Found Mass	Calc (M + 1)/1	Calc (M + 2)/2	Calc (M + 3)/3
SP378	Ac-ALTFs8EYWAQL\$AANleAA-NH2	387		1971.09	987.63	1972.1	986.55	658.04
SP379	Ac-AALTFs8EYWAQL\$AANleAA-NH2	388		2042.12	1022.69	2043.13	1022.07	681.71
SP380	Ac-LTFs8EYWAQCba\$AANleAA-NH2	389		1912.05	958.03	1913.06	957.03	638.36
SP381	Ac-LTFs8EYWAQhL\$AANleAA-NH2	390		1914.07	958.68	1915.08	958.04	639.03
SP382	Ac-ALTFs8EYWAQhL\$AANleAA-NH2	391		1985.1	994.1	1986.11	993.56	662.71
SP383	Ac-LTFs8ANmYWAQL\$AANleA-NH2	392		1785.02	894.11	1786.03	893.52	596.01
SP384	Ac-LTFs8ANmYWAQL\$AANleA-NH2	393	iso2	1785.02	894.11	1786.03	893.52	596.01
SP385	Ac-LTFs8AYNmWAQL\$AANleA-NH2	394		1785.02	894.11	1786.03	893.52	596.01
SP386	Ac-LTFs8AYNmWAQL\$AANleA-NH2	395	iso2	1785.02	894.11	1786.03	893.52	596.01
SP387	Ac-LTFs8AYAmwAQL\$AANleA-NH2	396		1785.02	894.01	1786.03	893.52	596.01
SP388	Ac-LTFs8AYAmwAQL\$AANleA-NH2	397	iso2	1785.02	894.01	1786.03	893.52	596.01
SP389	Ac-LTFs8AYWaiBQL\$AANleA-NH2	398		1785.02	894.01	1786.03	893.52	596.01
SP390	Ac-LTFs8AYWaiBQL\$AANleA-NH2	399	iso2	1785.02	894.01	1786.03	893.52	596.01
SP391	Ac-LTFs8AYWAQL\$AAibNleA-NH2	400		1785.02	894.38	1786.03	893.52	596.01
SP392	Ac-LTFs8AYWAQL\$AAibNleA-NH2	401	iso2	1785.02	894.38	1786.03	893.52	596.01
SP393	Ac-LTFs8AYWAQL\$AAaNleA-NH2	402		1771.01	887.54	1772.02	886.51	591.34
SP394	Ac-LTFs8AYWAQL\$AAaNleA-NH2	403	iso2	1771.01	887.54	1772.02	886.51	591.34
SP395	Ac-LTFs8AYWAQL\$ASarNleA-NH2	404		1771.01	887.35	1772.02	886.51	591.34
SP396	Ac-LTFs8AYWAQL\$ASarNleA-NH2	405	iso2	1771.01	887.35	1772.02	886.51	591.34
SP397	Ac-LTFs8AYWAQL\$AANleAib-NH2	406		1785.02	894.75	1786.03	893.52	596.01
SP398	Ac-LTFs8AYWAQL\$AANleAib-NH2	407	iso2	1785.02	894.75	1786.03	893.52	596.01
SP399	Ac-LTFs8AYWAQL\$AANleNmA-NH2	408		1785.02	894.6	1786.03	893.52	596.01
SP400	Ac-LTFs8AYWAQL\$AANleNmA-NH2	409	iso2	1785.02	894.6	1786.03	893.52	596.01
SP401	Ac-LTFs8AYWAQL\$AANleSar-NH2	410		1771.01	886.98	1772.02	886.51	591.34
SP402	Ac-LTFs8AYWAQL\$AANleSar-NH2	411	iso2	1771.01	886.98	1772.02	886.51	591.34
SP403	Ac-LTFs8AYWAQL\$AANleAAib-NH2	412		1856.06		1857.07	929.04	619.69
SP404	Ac-LTFs8AYWAQL\$AANleAAib-NH2	413	iso2	1856.06		1857.07	929.04	619.69
SP405	Ac-LTFs8AYWAQL\$AANleANmA-NH2	414		1856.06	930.37	1857.07	929.04	619.69
SP406	Ac-LTFs8AYWAQL\$AANleANmA-NH2	415	iso2	1856.06	930.37	1857.07	929.04	619.69
SP407	Ac-LTFs8AYWAQL\$AANleAa-NH2	416		1842.04	922.69	1843.05	922.03	615.02
SP408	Ac-LTFs8AYWAQL\$AANleAa-NH2	417	iso2	1842.04	922.69	1843.05	922.03	615.02
SP409	Ac-LTFs8AYWAQL\$AANleASar-NH2	418		1842.04	922.6	1843.05	922.03	615.02
SP410	Ac-LTFs8AYWAQL\$AANleASar-NH2	419	iso2	1842.04	922.6	1843.05	922.03	615.02
SP411	Ac-LTFs8AYWAQL\$AANleA-NH2	420		1799.04	901.14	1800.05	900.53	600.69
SP412	Ac-LTFs8AYWAQL\$AANleA-NH2	421		1648.9	826.02	1649.91	825.46	550.64
SP413	Ac-LTFs8Cou4YWAQL\$AANleA-NH2	422		1975.05	989.11	1976.06	988.53	659.36
SP414	Ac-LTFs8Cou4YWAQL\$AANleA-NH2	423	iso2	1975.05	989.11	1976.06	988.53	659.36
SP415	Ac-LTFs8AYWCou4QL\$AANleA-NH2	424		1975.05	989.11	1976.06	988.53	659.36
SP416	Ac-LTFs8AYWAQL\$Cou4ANleA-NH2	425		1975.05	989.57	1976.06	988.53	659.36
SP417	Ac-LTFs8AYWAQL\$Cou4ANleA-NH2	426	iso2	1975.05	989.57	1976.06	988.53	659.36
SP418	Ac-LTFs8AYWAQL\$ACou4NleA-NH2	427		1975.05	989.57	1976.06	988.53	659.36
SP419	Ac-LTFs8AYWAQL\$ACou4NleA-NH2	428	iso2	1975.05	989.57	1976.06	988.53	659.36
SP420	Ac-LTFs8AYWAQL\$AANleA-OH	429		1771.99	887.63	1773	887	591.67
SP421	Ac-LTFs8AYWAQL\$AANleA-OH	430	iso2	1771.99	887.63	1773	887	591.67
SP422	Ac-LTFs8AYWAQL\$AANleA-NHnPr	431		1813.05	908.08	1814.06	907.53	605.36
SP423	Ac-LTFs8AYWAQL\$AANleA-NHnPr	432	iso2	1813.05	908.08	1814.06	907.53	605.36
SP424	Ac-LTFs8AYWAQL\$AANleA-NHnBu33Me	433		1855.1	929.17	1856.11	928.56	619.37
SP425	Ac-LTFs8AYWAQL\$AANleA-NHnBu33Me	434	iso2	1855.1	929.17	1856.11	928.56	619.37
SP426	Ac-LTFs8AYWAQL\$AANleA-NHHex	435		1855.1	929.17	1856.11	928.56	619.37
SP427	Ac-LTFs8AYWAQL\$AANleA-NHHex	436	iso2	1855.1	929.17	1856.11	928.56	619.37
SP428	Ac-LTA8AYWAQL\$AANleA-NH2	437		1694.98	849.33	1695.99	848.5	566
SP429	Ac-LThL8AYWAQL\$AANleA-NH2	438		1751.04	877.09	1752.05	876.53	584.69
SP430	Ac-LTFs8AYAAQL\$AANleA-NH2	439		1655.97	829.54	1656.98	828.99	553
SP431	Ac-LTFs8AY2NalAQL\$AANleA-NH2	440		1782.01	892.63	1783.02	892.01	595.01
SP432	Ac-LTFs8EYWCou4QCba\$SAA-NH2	441		1947.97	975.8	1948.98	974.99	650.33
SP433	Ac-LTFs8EYWCou7QCba\$SAA-NH2	442		16.03	974.9	17.04	9.02	6.35
SP434	Ac-LTF%8EYWAQCba\$SAA-NH2	443		1745.94	874.8	1746.95	873.98	582.99
SP435	Dmaac-LTFs8EYWAQCba\$SAA-NH2	444		1786.97	894.8	1787.98	894.49	596.66
SP436	Dmaac-LTFs8AYWAQL\$AAAAAa-NH2	445		1914.08	958.2	1915.09	958.05	639.03
SP437	Dmaac-LTFs8AYWAQL\$AAAAAa-NH2	446	iso2	1914.08	958.2	1915.09	958.05	639.03
SP438	Dmaac-LTFs8EYWAQL\$AAAAAa-NH2	447		1972.08	987.3	1973.09	987.05	658.37
SP439	Dmaac-LTFs8EYWAQL\$AAAAAa-NH2	448	iso2	1972.08	987.3	1973.09	987.05	658.37
SP440	Dmaac-LTFs8EF4coohWAQCba\$AAIa-NH2	449		1912.05	957.4	1913.06	957.03	638.36
SP441	Dmaac-LTFs8EF4coohWAQCba\$AAIa-NH2	450	iso2	1912.05	957.4	1913.06	957.03	638.36
SP442	Dmaac-LTFs8AYWAQL\$AANleA-NH2	451		1814.05	908.3	1815.06	908.03	605.69
SP443	Dmaac-LTFs8AYWAQL\$AANleA-NH2	452	iso2	1814.05	908.3	1815.06	908.03	605.69
SP444	Ac-LTF%8AYWAQL%AANleA-NH2	453		1773.02	888.37	1774.03	887.52	592.01
SP445	Ac-LTF%8EYWAQL%AAAAAa-NH2	454		1931.06	966.4	1932.07	966.54	644.69
SP446	Cou6BaLTFs8EYWAQhL\$SAA-NH2	455		2018.05	1009.9	2019.06	1010.03	673.69
SP447	Cou8BaLTFs8EYWAQhL\$SAA-NH2	456		1962.96	982.34	1963.97	982.49	655.32
SP448	Ac-LTF4S8EYWAQL\$AAAAAa-NH2	457		2054.93	1028.68	2055.94	1028.47	685.98
SP449	Ac-LTFs8EYWAQL\$AAAAAa-NH2	458		1929.04	966.17	1930.05	965.53	644.02
SP550	Ac-LTFs8EYWAQL\$AAAAAa-OH	459		1930.02	966.54	1931.03	966.02	644.35
SP551	Ac-LTFs8EYWAQL\$AAAAAa-OH	460	iso2	1930.02	965.89	1931.03	966.02	644.35
SP552	Ac-LTFs8EYWAEL\$AAAAAa-NH2	461		1930.02	966.82	1931.03	966.02	644.35
SP553	Ac-LTFs8EYWAEL\$AAAAAa-NH2	462	iso2	1930.02	966.91	1931.03	966.02	644.35

TABLE 1-continued

SP	Sequence	SEQ ID NO:	Isomer	Exact Mass	Found Mass	Calc (M + 1)/1	Calc (M + 2)/2	Calc (M + 3)/3
SP554	Ac-LTF\$8EYWAEL\$AAAAAa-OH	463		1931.01	967.28	1932.02	966.51	644.68
SP555	Ac-LTF\$8EY6cIWAQL\$AAAAAa-NH2	464		1963	983.28	1964.01	982.51	655.34
SP556	Ac-LTF\$8EF4bOH2WAQL\$AAAAAa-NH2	465		1957.05	980.04	1958.06	979.53	653.36
SP557	Ac-AAALTF\$8EYWAQL\$AAAAAa-NH2	466		2142.15	1072.83	2143.16	1072.08	715.06
SP558	Ac-LTF34F2\$8EYWAQL\$AAAAAa-NH2	467		1965.02	984.3	1966.03	983.52	656.01
SP559	Ac-RTF\$8EYWAQL\$AAAAAa-NH2	468		1972.06	987.81	1973.07	987.04	658.36
SP560	Ac-LTA\$8EYWAQL\$AAAAAa-NH2	469		1853.01	928.33	1854.02	927.51	618.68
SP561	Ac-LTF\$8EYWAibQL\$AAAAAa-NH2	470		1943.06	973.48	1944.07	972.54	648.69
SP562	Ac-LTF\$8EYWAQL\$AAiAaAa-NH2	471		1943.06	973.11	1944.07	972.54	648.69
SP563	Ac-LTF\$8EYWAQL\$AAiAaAa-NH2	472		1943.06	973.48	1944.07	972.54	648.69
SP564	Ac-LTF\$8EYWAQL\$AAiAaAa-NH2	473		1943.06	973.48	1944.07	972.54	648.69
SP565	Ac-LTF\$8EYWAQL\$AAiAaAa-NH2	474		1943.06	973.38	1944.07	972.54	648.69
SP566	Ac-LTF\$8EYWAQL\$AAiAaAa-NH2	475	iso2	1943.06	973.38	1944.07	972.54	648.69
SP567	Ac-LTF\$8EYWAQL\$AAiAaAa-NH2	476		1943.06	973.01	1944.07	972.54	648.69
SP568	Ac-LTF\$8EYWAQL\$AAiAaAa-NH2	477		1929.04	966.54	1930.05	965.53	644.02
SP569	Ac-LTF\$8EYWAQL\$AAiAaAa-NH2	478		1929.04	966.35	1930.05	965.53	644.02
SP570	Ac-LTF\$8EYWAQL\$AAiAaAa-NH2	479		1929.04	966.54	1930.05	965.53	644.02
SP571	Ac-LTF\$8EYWAQL\$AAiAaAa-NH2	480	iso2	1929.04	966.35	1930.05	965.53	644.02
SP572	Ac-LTF\$8EYWAQL\$AAiAaAa-NH2	481		1929.04	966.35	1930.05	965.53	644.02
SP573	Ac-LTF\$8EYWAQL\$AAiAaAa-NH2	482		1929.04	966.35	1930.05	965.53	644.02
SP574	Ac-LTF\$8EYWAQL\$ASarAaAa-NH2	483		1929.04	966.54	1930.05	965.53	644.02
SP575	Ac-LTF\$8EYWAQL\$ASarAaAa-NH2	484		1929.04	966.35	1930.05	965.53	644.02
SP576	Ac-LTF\$8EYWAQL\$ASarAaAa-NH2	485		1929.04	966.35	1930.05	965.53	644.02
SP577	Ac-LTF\$8EYWAQL\$ASarAaAa-NH2	486		1929.04	966.35	1930.05	965.53	644.02
SP578	Ac-LTF\$8EYWAQL\$ASarAaAa-NH2	487		1929.04	966.08	1930.05	965.53	644.02
SP579	Ac-7LTF\$8EYWAQL\$AAAAAa-NH2	488		1918.07	951.99	1919.08	960.04	640.37
SP581	Ac-TF\$8EYWAQL\$AAAAAa-NH2	489		1815.96	929.85	1816.97	908.99	606.33
SP582	Ac-F\$8EYWAQL\$AAAAAa-NH2	490		1714.91	930.92	1715.92	858.46	572.64
SP583	Ac-LV\$8EYWAQL\$AAAAAa-NH2	491		1927.06	895.12	1928.07	964.54	643.36
SP584	Ac-AAF\$8EYWAQL\$AAAAAa-NH2	492		1856.98	859.51	1857.99	929.5	620
SP585	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	493		1858	824.08	1859.01	930.01	620.34
SP586	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	494		1786.97	788.56	1787.98	894.49	596.66
SP587	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	495		1715.93	1138.57	1716.94	858.97	572.98
SP588	Ac-LTF\$8EYWAQL\$Aa-NH2	496		1644.89	1144.98	1645.9	823.45	549.3
SP589	Ac-LTF\$8EYWAQL\$Aa-NH2	497		1573.85	1113.71	1574.86	787.93	525.62
SP590	Ac-LTF\$8EYWAQL\$AAAA-OH	498		1716.91	859.55	1717.92	859.46	573.31
SP591	Ac-LTF\$8EYWAQL\$A-OH	499		1574.84	975.14	1575.85	788.43	525.95
SP592	Ac-LTF\$8EYWAQL\$AAAA-NH2	500		1715.93	904.75	1716.94	858.97	572.98
SP593	Ac-LTF\$8EYWAQL\$Cba\$Aa-OH	501		1744.91	802.49	1745.92	873.46	582.64
SP594	Ac-LTF\$8EYWAQL\$Cba\$S-OH	502		1602.83	913.53	1603.84	802.42	535.28
SP595	Ac-LTF\$8EYWAQL\$Cba\$S-NH2	503		1601.85	979.58	1602.86	801.93	534.96
SP596	4-FBzl-LTF\$8EYWAQL\$AAAAAa-NH2	504		2009.05	970.52	2010.06	1005.53	670.69
SP597	4-FBzl-LTF\$8EYWAQL\$Cba\$SAA-NH2	505		1823.93	965.8	1824.94	912.97	608.98
SP598	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	506		1956.1	988.28	1957.11	979.06	653.04
SP599	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	507		1937.06	1003.54	1938.07	969.54	646.69
SP600	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	508		1928.06	993.92	1929.07	965.04	643.69
SP601	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	509		1957.08	987	1958.09	979.55	653.37
SP602	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	510		1973.03	983	1974.04	987.52	658.68
SP603	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	511		2004.1	937.86	2005.11	1003.06	669.04
SP604	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	512		1984.13	958.58	1985.14	993.07	662.38
SP605	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	513		1970.14	944.52	1971.15	986.08	657.72
SP606	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	514		1962.08	946	1963.09	982.05	655.03
SP607	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	515		1872.02	959.32	1873.03	937.02	625.01
SP608	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	516		1914.07	980.88	1915.08	958.04	639.03
SP609	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	517		1886.03	970.61	1887.04	944.02	629.68
SP610	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	518		1888.01	980.51	1889.02	945.01	630.34
SP611	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	519		1915.02	1006.41	1916.03	958.52	639.35
SP612	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	520		1958.07		1959.08	980.04	653.7
SP613	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	521		1938.04	966.24	1939.05	970.03	647.02
SP614	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	522		1957.08		1958.09	979.55	653.37
SP615	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	523		2009.01		2010.02	1005.51	670.68
SP616	Cbm-LTF\$8EYWAQL\$AAAAAa-NH2	524		1590.85		1591.86	796.43	531.29
SP617	Cbm-LTF\$8EYWAQL\$AAAAAa-NH2	525		1930.04		1931.05	966.03	644.35
SP618	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	526		1945.04	1005.11	1946.05	973.53	649.35
SP619	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	527		1945.04	986.52	1946.05	973.53	649.35
SP620	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	528		1961.03	993.27	1962.04	981.52	654.68
SP621	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	529		1943.06	983.1	1944.07	972.54	648.69
SP622	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	530		2007.09	990.31	2008.1	1004.55	670.04
SP623	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	531		1969.07	987.17	1970.08	985.54	657.36
SP624	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	532		1983.09	1026.11	1984.1	992.55	662.04
SP625	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	533		1963.02	957.01	1964.03	982.52	655.35
SP626	Ac-LTF\$8EYWAQL\$AAAAAa-NH2	534		1977.04	1087.81	1978.05	989.53	660.02
SP627	Ac-LTF\$8EYWAQL\$AAiAaAa-NH2	535		1971.09	933.45	1972.1	986.55	658.04
SP628	Ac-LTF\$8EYWAQL\$AAiAaAa-NH2	536		2049.13	1017.97	2050.14	1025.57	684.05
SP629	4-FBz-BaLTF\$8EYWAQL\$AAAAAa-NH2	537		2080.08		2081.09	1041.05	694.37
SP630	4-FBz-BaLTF\$8EYWAQL\$AAAAAa-NH2	538		1894.97		1895.98	948.49	632.66

TABLE 1-continued

SP	Sequence	SEQ ID NO:	Isomer	Exact Mass	Found Mass	Calc (M + 1)/1	Calc (M + 2)/2	Calc (M + 3)/3
SP631	Ac-LTF\$r5EYWAQL\$s8AAAAAa-NH2	539		1929.04	1072.68	1930.05	965.53	644.02
SP632	Ac-LTF\$r5EYWAQCba\$s8SAA-NH2	540		1743.92	1107.79	1744.93	872.97	582.31
SP633	Ac-LTF\$r8EYWAQL\$AAAhLAAa-NH2	541		1999.12		2000.13	1000.57	667.38
SP634	Ac-LTF\$r8EYWAQL\$AAAAAAa-NH2	542		2071.11		2072.12	1036.56	691.38
SP635	Ac-LTF\$r8EYWAQL\$AAAAAAa-NH2	543		2142.15	778.1	2143.16	1072.08	715.06
SP636	Ac-LTF\$r8EYWAQL\$AAAAAAa-NH2	544		2213.19	870.53	2214.2	1107.6	738.74
SP637	Ac-LTA\$r8EYAAQCba\$SAA-NH2	545		1552.85		1553.86	777.43	518.62
SP638	Ac-LTA\$r8EYAAQL\$AAAAAa-NH2	546		1737.97	779.45	1738.98	869.99	580.33
SP639	Ac-LTF\$r8EPmpWAQL\$AAAAAa-NH2	547		2007.03	779.54	2008.04	1004.52	670.02
SP640	Ac-LTF\$r8EPmpWAQCba\$SAA-NH2	548		1821.91	838.04	1822.92	911.96	608.31
SP641	Ac-ATF\$r8HYWAQL\$S-NH2	549		1555.82	867.83	1556.83	778.92	519.61
SP642	Ac-LTF\$r8HAWAQL\$S-NH2	550		1505.84	877.91	1506.85	753.93	502.95
SP643	Ac-LTF\$r8HYWAQAS\$S-NH2	551		1555.82	852.52	1556.83	778.92	519.61
SP644	Ac-LTF\$r8EYWAQCba\$SAA-NH2	552		1672.89	887.18	1673.9	837.45	558.64
SP645	Ac-LTF\$r8EYWAQCba\$SAA-NH2	553		1731.92	873.32	1732.93	866.97	578.31
SP646	Ac-LTF\$r8HYWAQCba\$SAA-NH2	554		1751.94	873.05	1752.95	876.98	584.99
SP647	Ac-LTF\$r8SYWAQCba\$SAA-NH2	555		1701.91	844.88	1702.92	851.96	568.31
SP648	Ac-LTF\$r8RYWAQCba\$SAA-NH2	556		1770.98	865.58	1771.99	886.5	591.33
SP649	Ac-LTF\$r8KYWAQCba\$SAA-NH2	557		1742.98	936.57	1743.99	872.5	582
SP650	Ac-LTF\$r8QYWAQCba\$SAA-NH2	558		1742.94	930.93	1743.95	872.48	581.99
SP651	Ac-LTF\$r8EYWAQCba\$SAA-NH2	559		1686.9	1032.45	1687.91	844.46	563.31
SP652	Ac-LTF\$r8EYWAQCba\$AAA-NH2	560		1727.93	895.46	1728.94	864.97	576.98
SP653	Ac-LTF\$r8EYWAQL\$AAAAA-OH	561		1858.99	824.54	1860	930.5	620.67
SP654	Ac-LTF\$r8EYWAQL\$AAAAA-OH	562		1787.95	894.48	1788.96	894.98	596.99
SP655	Ac-LTF\$r8EYWAQL\$AAA-OH	563		1645.88	856	1646.89	823.95	549.63
SP656	Ac-LTF\$r8AF4bOH2WAQL\$AAAAAa-NH2	564						
SP657	Ac-LTF\$r8AF4bOH2WAAL\$AAAAAa-NH2	565						
SP658	Ac-LTF\$r8EF4bOH2WAQCba\$SAA-NH2	566						
SP659	Ac-LTF\$r8ApYWAQL\$AAAAAa-NH2	567						
SP660	Ac-LTF\$r8ApYWAAL\$AAAAAa-NH2	568						
SP661	Ac-LTF\$r8EpYWAQCba\$SAA-NH2	569						
SP662	Ac-LTF\$rda6AYWAQL\$da5AAAAAa-NH2	570		1974.06	934.44			
SP663	Ac-LTF\$rda6EYWAQCba\$da5SAA-NH2	571		1846.95	870.52		869.94	
SP664	Ac-LTF\$rda6EYWAQL\$da5AAAAAa-NH2	572						
SP665	Ac-LTF\$ra9EYWAQL\$sa6AAAAAa-NH2	573			936.57		935.51	
SP666	Ac-LTF\$ra9EYWAQL\$sa6AAAAAa-NH2	574						
SP667	Ac-LTF\$ra9EYWAQCba\$sa6SAA-NH2	575						
SP668	Ac-LTA\$ra9EYWAQCba\$sa6SAA-NH2	576						
SP669	5-FAM-BaLTF\$r8EYWAQCba\$sa6SAA-NH2	577						
SP670	5-FAM-BaLTF\$r8EYWAQL\$AAAAAa-NH2	578		2316.11				
SP671	5-FAM-BaLTF\$r8EYWAQL\$/AAAAAa-NH2	579		2344.15				
SP672	5-FAM-BaLTA\$r8EYWAQL\$AAAAAa-NH2	580		2240.08				
SP673	5-FAM-BaLTF\$r8AYWAQL\$AAAAAa-NH2	581		2258.11				
SP674	5-FAM-BaATF\$r8EYWAQL\$AAAAAa-NH2	582		2274.07				
SP675	5-FAM-BaLAF\$r8EYWAQL\$AAAAAa-NH2	583		2286.1				
SP676	5-FAM-BaLTF\$r8EAWAQL\$AAAAAa-NH2	584		2224.09				
SP677	5-FAM-BaLTF\$r8EYAAQL\$AAAAAa-NH2	585		2201.07				
SP678	5-FAM-BaLTA\$r8EYAAQL\$AAAAAa-NH2	586		2125.04				
SP679	5-FAM-BaLTF\$r8EYWAAL\$AAAAAa-NH2	587		2259.09				
SP680	5-FAM-BaLTF\$r8EYWAQAS\$AAAAAa-NH2	588		2274.07				
SP681	5-FAM-BaLTF\$r8EYWAQCba\$/SAA-NH2	589		2159.03				
SP682	5-FAM-BaLTA\$r8EYWAQCba\$SAA-NH2	590		2054.97				
SP683	5-FAM-BaLTF\$r8EYAAQCba\$SAA-NH2	591		2015.96				
SP684	5-FAM-BaLTA\$r8EYAAQCba\$SAA-NH2	592		1939.92				
SP685	5-FAM-BaQSQTf\$r8NLWRL\$QN-NH2	593		2495.23				
SP686	5-TAMRA-BaLTF\$r8EYWAQCba\$SAA-NH2	594		2186.1				
SP687	5-TAMRA-BaLTA\$r8EYWAQCba\$SAA-NH2	595		2110.07				
SP688	5-TAMRA-BaLTF\$r8EYAAQCba\$SAA-NH2	596		2071.06				
SP689	5-TAMRA-BaLTA\$r8EYAAQCba\$SAA-NH2	597		1995.03				
SP690	5-TAMRA-BaLTF\$r8EYWAQCba\$/SAA-NH2	598		2214.13				
SP691	5-TAMRA-BaLTF\$r8EYWAQL\$AAAAAa-NH2	599		2371.22				
SP692	5-TAMRA-BaLTA\$r8EYWAQL\$AAAAAa-NH2	600		2295.19				
SP693	5-TAMRA-BaLTF\$r8EYWAQL\$/AAAAAa-NH2	601		2399.25				
SP694	Ac-LTF\$r8EYWCou7QCba\$SAA-OH	602		1947.93				
SP695	Ac-LTF\$r8EYWCou7QCba\$S-OH	603		1805.86				
SP696	Ac-LTA\$r8EYWCou7QCba\$SAA-NH2	604		1870.91				
SP697	Ac-LTF\$r8EYACou7QCba\$SAA-NH2	605		1831.9				
SP698	Ac-LTA\$r8EYACou7QCba\$SAA-NH2	606		1755.87				
SP699	Ac-LTF\$r8EYWCou7QCba\$/SAA-NH2	607		1974.98				
SP700	Ac-LTF\$r8EYWCou7QL\$AAAAAa-NH2	608		2132.06				
SP701	Ac-LTF\$/r8EYWCou7QL\$/AAAAAa-NH2	609		2160.09				
SP702	Ac-LTF\$r8EYWCou7QL\$AAAAA-OH	610		2062.01				
SP703	Ac-LTF\$r8EYWCou7QL\$AAAAA-OH	611		1990.97				
SP704	Ac-LTF\$r8EYWCou7QL\$AAA-OH	612		1919.94				
SP705	Ac-LTF\$r8EYWCou7QL\$AA-OH	613		1848.9				
SP706	Ac-LTF\$r8EYWCou7QL\$A-OH	614		1777.86				

TABLE 1-continued

SP	Sequence	SEQ ID NO:	Isomer	Exact Mass	Found Mass	Calc (M + 1)/1	Calc (M + 2)/2	Calc (M + 3)/3
SP707	Ac-LTFs8EYWAQL\$AAAAAa-NH2	615	iso2		974.4		973.53	
SP708	Ac-LTFs8AYWAAL\$AAAAAa-NH2	616	iso2	1814.01	908.82	1815.02	908.01	605.68
SP709	Biotin-BaLTFs8EYWAQL\$AAAAAa-NH2	617		2184.14	1093.64	2185.15	1093.08	729.05
SP710	Ac-LTFs8HAWAQL\$S-NH2	618	iso2	1505.84	754.43	1506.85	753.93	502.95
SP711	Ac-LTFs8EYWAQCba\$SAA-NH2	619	iso2	1672.89	838.05	1673.9	837.45	558.64
SP712	Ac-LTFs8HYWAQCba\$SAA-NH2	620	iso2	1751.94	877.55	1752.95	876.98	584.99
SP713	Ac-LTFs8SYWAQCba\$SAA-NH2	621	iso2	1701.91	852.48	1702.92	851.96	568.31
SP714	Ac-LTFs8RYWAQCba\$SAA-NH2	622	iso2	1770.98	887.45	1771.99	886.5	591.33
SP715	Ac-LTFs8KYWAQCba\$SAA-NH2	623	iso2	1742.98	872.92	1743.99	872.5	582
SP716	Ac-LTFs8EYWAQCba\$AAA-NH2	624	iso2	1727.93	865.71	1728.94	864.97	576.98
SP717	Ac-LTFs8EYWAQL\$AAAAAaBaC-NH2	625		2103.09	1053.12	2104.1	1052.55	702.04
SP718	Ac-LTFs8EYWAQL\$AAAAAadPeg4C-NH2	626		2279.19	1141.46	2280.2	1140.6	760.74
SP719	Ac-LTA\$8AYWAAL\$AAAAAa-NH2	627		1737.98	870.43	1738.99	870	580.33
SP720	Ac-LTFs8AYAAAL\$AAAAAa-NH2	628		1698.97	851	1699.98	850.49	567.33
SP721	5-FAM-BaLTFs8AYWAAL\$AAAAAa-NH2	629		2201.09	1101.87	2202.1	1101.55	734.7
SP722	Ac-LTA\$8AYWAQL\$AAAAAa-NH2	630		1795	898.92	1796.01	898.51	599.34
SP723	Ac-LTFs8AYAAQL\$AAAAAa-NH2	631		1755.99	879.49	1757	879	586.34
SP724	Ac-LTFs8da6AYWAAL\$da5AAAAAa-NH2	632		1807.97		1808.98	904.99	603.66
SP725	FITC-BaLTFs8EYWAQL\$AAAAAa-NH2	633		2347.1	1174.49	2348.11	1174.56	783.37
SP726	FITC-BaLTFs8EYWAQCba\$SAA-NH2	634		2161.99	1082.35	2163	1082	721.67
SP733	Ac-LTFs8EYWAQL\$AAAAAa-NH2	635		1987.05	995.03	1988.06	994.53	663.36
SP734	Ac-LTFs8AYWAQL\$AAAAAa-NH2	636		1929.04	966.35	1930.05	965.53	644.02
SP735	Ac-LTFs8EYWAQL\$AAAAAaBaKbio-NH2	637		2354.25	1178.47	2355.26	1178.13	785.76
SP736	Ac-LTFs8AYWAAL\$AAAAAa-NH2	638		1814.01	908.45	1815.02	908.01	605.68
SP737	Ac-LTFs8AYAAAL\$AAAAAa-NH2	639	iso2	1698.97	850.91	1699.98	850.49	567.33
SP738	Ac-LTFs8AYAAQL\$AAAAAa-NH2	640	iso2	1755.99	879.4	1757	879	586.34
SP739	Ac-LTFs8EYWAQL\$AAAAAa-NH2	641	iso2	1987.05	995.21	1988.06	994.53	663.36
SP740	Ac-LTFs8AYWAQL\$AAAAAa-NH2	642	iso2	1929.04	966.08	1930.05	965.53	644.02
SP741	Ac-LTFs8EYWAQCba\$SAAAAa-NH2	643		1957.04	980.04	1958.05	979.53	653.35
SP742	Ac-LTFs8EYWAQL\$StAAA\$5AA-NH2	644		2023.12	1012.83	2024.13	1012.57	675.38
SP743	Ac-LTFs8EYWAQL\$A\$AAA\$A-NH2	645		2108.17	1055.44	2109.18	1055.09	703.73
SP744	Ac-LTFs8EYWAQL\$AA\$AAA\$A-NH2	646		2179.21	1090.77	2180.22	1090.61	727.41
SP745	Ac-LTFs8EYWAQL\$AA\$AAA\$A-NH2	647		2250.25	1126.69	2251.26	1126.13	751.09
SP746	Ac-AAALTFs8EYWAQL\$AAA-OH	648		1930.02		1931.03	966.02	644.35
SP747	Ac-AAALTFs8EYWAQL\$AAA-NH2	649		1929.04	965.85	1930.05	965.53	644.02
SP748	Ac-AAALTFs8EYWAQL\$AAA-NH2	650		2000.08	1001.4	2001.09	1001.05	667.7
SP749	Ac-AAALTFs8EYWAQL\$AAA-NH2	651		2071.11	1037.13	2072.12	1036.56	691.38
SP750	Ac-AAALTFs8EYWAQL\$AAA-NH2	652		2142.15		2143.16	1072.08	715.06
SP751	Ac-LTFs8da6EYWAQCba\$da6SAA-NH2	653	iso2	1751.89	877.36	1752.9	876.95	584.97
SP752	Ac-t\$5wya\$5f4CF3ekllr-NH2	654			844.25			
SP753	Ac-tawy\$5nf4CF3e\$5lr-NH2	655			837.03			
SP754	Ac-tawya\$5f4CF3ek\$5lr-NH2	656			822.97			
SP755	Ac-tawyanf4CF3e\$5lr\$5a-NH2	657			908.35			
SP756	Ac-t\$8wyanf4CF3e\$5lr-NH2	658			858.03			
SP757	Ac-tawy\$8nf4CF3ekllr\$5a-NH2	659			879.86			
SP758	Ac-tawya\$8f4CF3ekllr\$5a-NH2	660			936.38			
SP759	Ac-tawy\$8naekllr\$5a-NH2	661			844.25			
SP760	5-FAM-Batawy\$8nf4CF3ekllr\$5a-NH2	662						
SP761	5-FAM-Batawy\$8naekllr\$5a-NH2	663						
SP762	Ac-tawy\$8nf4CF3eallr\$5a-NH2	664						
SP763	Ac-tawy\$8nf4CF3ekllr\$5aaaa-NH2	665						
SP764	Ac-tawy\$8nf4CF3eallr\$5aaaa-NH2	666						

Table 1a shows a selection of peptidomimetic macro-cycles.

TABLE 1a

SP	Sequence	SEQ ID NO:	Isomer	Exact Mass	Found Mass	Calc (M + 1)/1	Calc (M + 2)/2	Calc (M + 3)/3
SP244	Ac-LTFs8EF4coohWAQCba\$SANleA-NH2	667		1885	943.59	1886.01	943.51	629.34
SP331	Ac-LTFs8EYWAQL\$AAAAAa-NH2	668	iso2	1929.04	966.08	1930.05	965.53	644.02
SP555	Ac-LTFs8EY6elWAQL\$AAAAAa-NH2	669		1963	983.28	1964.01	982.51	655.34
SP557	Ac-AAALTFs8EYWAQL\$AAAAAa-NH2	670		2142.15	1072.83	2143.16	1072.08	715.06
SP558	Ac-LTF34F2\$8EYWAQL\$AAAAAa-NH2	671		1965.02	984.3	1966.03	983.52	656.01
SP562	Ac-LTFs8EYWAQL\$AAIbAAAAa-NH2	672		1943.06	973.11	1944.07	972.54	648.69
SP564	Ac-LTFs8EYWAQL\$AAAAIbAa-NH2	673		1943.06	973.48	1944.07	972.54	648.69
SP566	Ac-LTFs8EYWAQL\$AAAAIbAa-NH2	674	iso2	1943.06	973.38	1944.07	972.54	648.69
SP567	Ac-LTFs8EYWAQL\$AAAAAIb-NH2	675		1943.06	973.01	1944.07	972.54	648.69
SP572	Ac-LTFs8EYWAQL\$AAAAAa-NH2	676		1929.04	966.35	1930.05	965.53	644.02
SP573	Ac-LTFs8EYWAQL\$AAAAAa-NH2	677		1929.04	966.35	1930.05	965.53	644.02

TABLE 1a-continued

SP	Sequence	SEQ ID NO:	Isomer	Exact Mass	Found Mass	Calc (M + 1)/1	Calc (M + 2)/2	Calc (M + 3)/3
SP578	Ac-LTF\$R8EYWAQL\$AAAAASar-NH2	678		1929.04	966.08	1930.05	965.53	644.02
SP551	Ac-LTF\$R8EYWAQL\$AAAAAa-OH	679	iso2	1930.02	965.89	1931.03	966.02	644.35
SP662	Ac-LTF\$Rda6AYWAQL\$da5AAAAa-NH2	680		1974.06	934.44		933.49	
SP367	5-FAM-BaLTF\$R8EYWAQCba\$AAA-NH2	681		2131	1067.09	2132.01	1066.51	711.34
SP349	Ac-LTF\$R8EF4coohWAQCba\$AAAAAa-NH2	682	iso2	1969.04	986.06	1970.05	985.53	657.35
SP347	Ac-LTF\$R8EYWAQCba\$AAAAAa-NH2	683	iso2	1941.04	972.55	1942.05	971.53	648.02

Table 1b shows a selection of peptidomimetic macrocycles.

olefin side chains that are not crosslinked. Amino acids represented as “% St” are amino acids comprising two

TABLE 1b

SP	Sequence	SEQ ID NO:	Isomer	Exact Mass	Found Mass	Calc (M + 1)/1	Calc (M + 2)/2	Calc (M + 3)/3
SP581	Ac-TF\$R8EYWAQL\$AAAAAa-NH2	684		1815.96	929.85	1816.97	908.99	606.33
SP582	Ac-F\$R8EYWAQL\$AAAAAa-NH2	685		1714.91	930.92	1715.92	858.46	572.64
SP583	Ac-LVF\$R8EYWAQL\$AAAAAa-NH2	686		1927.06	895.12	1928.07	964.54	643.36
SP584	Ac-AAF\$R8EYWAQL\$AAAAAa-NH2	687		1856.98	859.51	1857.99	929.5	620
SP585	Ac-LTF\$R8EYWAQL\$AAAAAa-NH2	688		1858	824.08	1859.01	930.01	620.34
SP586	Ac-LTF\$R8EYWAQL\$AAAAa-NH2	689		1786.97	788.56	1787.98	894.49	596.66
SP587	Ac-LTF\$R8EYWAQL\$AAAa-NH2	690		1715.93	1138.57	1716.94	858.97	572.98
SP588	Ac-LTF\$R8EYWAQL\$Aa-NH2	691		1644.89	1144.98	1645.9	823.45	549.3
SP589	Ac-LTF\$R8EYWAQL\$a-NH2	692		1573.85	1113.71	1574.86	787.93	525.62

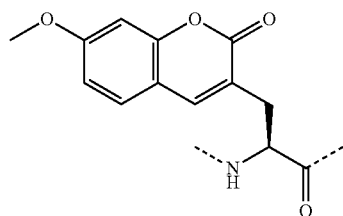
In the sequences shown above and elsewhere, the following abbreviations are used: “Nle” represents norleucine, “Aib” represents 2-aminoisobutyric acid, “Ac” represents acetyl, and “Pr” represents propionyl. Amino acids represented as “\$” are alpha-Me S5-pentenyl-alanine olefin amino acids connected by an all-carbon crosslinker comprising one double bond. Amino acids represented as “\$r5” are alpha-Me R5-pentenyl-alanine olefin amino acids connected by an all-carbon comprising one double bond. Amino acids represented as “\$s8” are alpha-Me S8-octenyl-alanine olefin amino acids connected by an all-carbon crosslinker comprising one double bond. Amino acids represented as “\$r8” are alpha-Me R8-octenyl-alanine olefin amino acids connected by an all-carbon crosslinker comprising one double bond. “Ahx” represents an aminocyclohexyl linker. The crosslinkers are linear all-carbon crosslinker comprising eight or eleven carbon atoms between the alpha carbons of each amino acid. Amino acids represented as “\$/” are alpha-Me S5-pentenyl-alanine olefin amino acids that are not connected by any crosslinker. Amino acids represented as “\$/r5” are alpha-Me R5-pentenyl-alanine olefin amino acids that are not connected by any crosslinker. Amino acids represented as “\$/s8” are alpha-Me S8-octenyl-alanine olefin amino acids that are not connected by any crosslinker. Amino acids represented as “\$/r8” are alpha-Me R8-octenyl-alanine olefin amino acids that are not connected by any crosslinker. Amino acids represented as “Amw” are alpha-Me tryptophan amino acids. Amino acids represented as “Aml” are alpha-Me leucine amino acids. Amino acids represented as “Amf” are alpha-Me phenylalanine amino acids. Amino acids represented as “2ff” are 2-fluoro-phenylalanine amino acids. Amino acids represented as “3ff” are 3-fluoro-phenylalanine amino acids. Amino acids represented as “St” are amino acids comprising two pentenyl-alanine olefin side chains, each of which is crosslinked to another amino acid as indicated. Amino acids represented as “St/” are amino acids comprising two pentenyl-alanine

30 pentenyl-alanine olefin side chains, each of which is crosslinked to another amino acid as indicated via fully saturated hydrocarbon crosslinks. Amino acids represented as “Ba” are beta-alanine. The lower-case character “e” or “z” within the designation of a crosslinked amino acid (e.g. “Ser8” or “\$zr8”) represents the configuration of the double bond (E or Z, respectively). In other contexts, lower-case letters such as “a” or “f” represent D amino acids (e.g. D-alanine, or D-phenylalanine, respectively). Amino acids designated as “NmW” represent N-methyltryptophan. Amino acids designated as “NmY” represent N-methyltyrosine. Amino acids designated as “NmA” represent N-methylalanine. “Kbio” represents a biotin group attached to the side chain amino group of a lysine residue. Amino acids designated as “Sar” represent sarcosine. Amino acids designated as “Cha” represent cyclohexyl alanine. Amino acids designated as “Cpg” represent cyclopentyl glycine. Amino acids designated as “Chg” represent cyclohexyl glycine. Amino acids designated as “Cba” represent cyclobutyl alanine. Amino acids designated as “F4I” represent 4-iodo phenylalanine. “7L” represents N15 isotopic leucine. Amino acids designated as “F3Cl” represent 3-chloro phenylalanine. Amino acids designated as “F4cooh” represent 4-carboxy phenylalanine. Amino acids designated as “F34F2” represent 3,4-difluoro phenylalanine. Amino acids designated as “6clW” represent 6-chloro tryptophan. Amino acids designated as “\$Rda6” represent alpha-Me R6-hexynyl-alanine alkynyl amino acids, crosslinked via a dialkyne bond to a second alkynyl amino acid. Amino acids designated as “\$da5” represent alpha-Me S5-pentenyl-alanine alkynyl amino acids, wherein the alkyne forms one half of a dialkyne bond with a second alkynyl amino acid. Amino acids designated as “\$ra9” represent alpha-Me R9-nonyl-alanine alkynyl amino acids, crosslinked via an alkyne metathesis reaction with a second alkynyl amino acid. Amino acids designated as “\$a6” represent alpha-Me S6-hexynyl-alanine alkynyl amino acids, crosslinked via an alkyne metathesis reaction

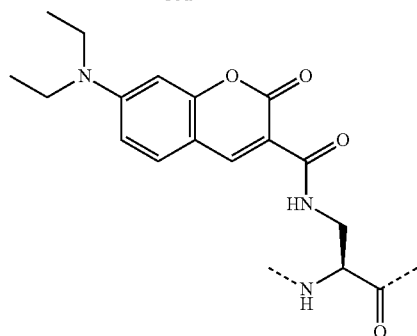
83

with a second alkynyl amino acid. The designation “iso1” or “iso2” indicates that the peptidomimetic macrocycle is a single isomer.

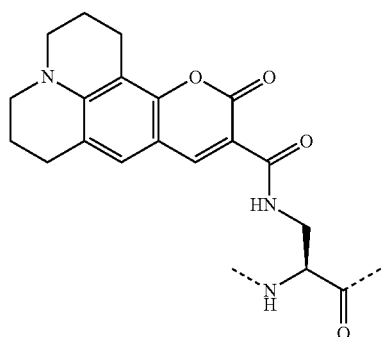
Amino acids designated as “Cit” represent citrulline. Amino acids designated as “Cou4”, “Cou6”, “Cou7” and “Cou8”, respectively, represent the following structures:



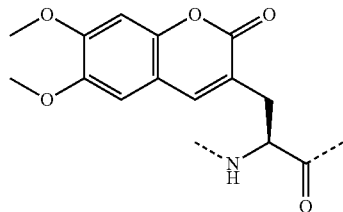
Cou



Cou2



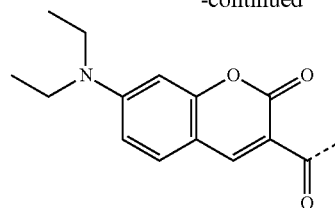
Cou3



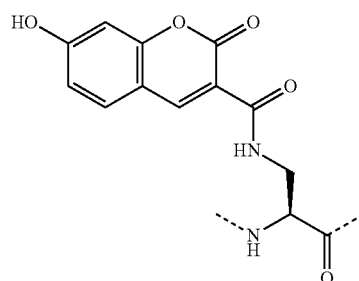
Cou4

84

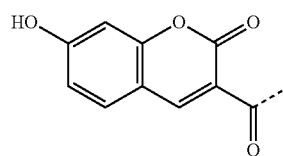
-continued



Cou6



Cou7



Cou8

In some embodiments, a peptidomimetic macrocycle is obtained in more than one isomer, for example due to the configuration of a double bond within the structure of the crosslinker (E vs Z). Such isomers can or can not be separable by conventional chromatographic methods. In some embodiments, one isomer has improved biological properties relative to the other isomer. In one embodiment, an E crosslinker olefin isomer of a peptidomimetic macrocycle has better solubility, better target affinity, better in vivo or in vitro efficacy, higher helicity, or improved cell permeability relative to its Z counterpart. In another embodiment, a Z crosslinker olefin isomer of a peptidomimetic macrocycle has better solubility, better target affinity, better in vivo or in vitro efficacy, higher helicity, or improved cell permeability relative to its E counterpart.

Table 1c shows exemplary peptidomimetic macrocycle:

TABLE 1c

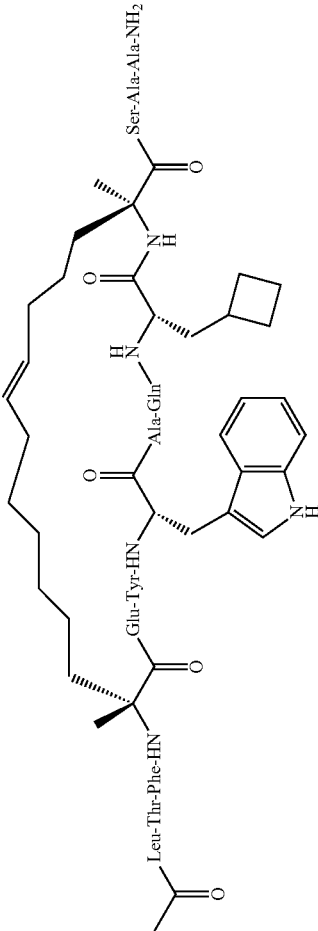
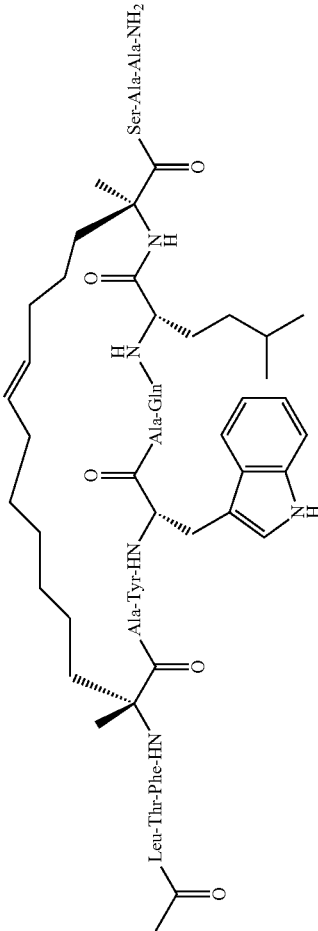
Structure	
SP154 (SEQ ID NO: 163)  Ac-L T F Ser8EYWAQCha \$e\$ SAA -NH₂ Chemical Formula: C₈₈H₁₂₅N₁₇O₂₁ Exact Mass: 1743.92 Molecular Weight: 1745.02	SP115 (SEQ ID NO: 124)  Ac-L T F Ser8AYWAQhL \$e\$ SAA -NH₂ Chemical Formula: C₈₈H₁₂₅N₁₇O₁₉ Exact Mass: 1687.93 Molecular Weight: 1689.00

TABLE 1c-continued

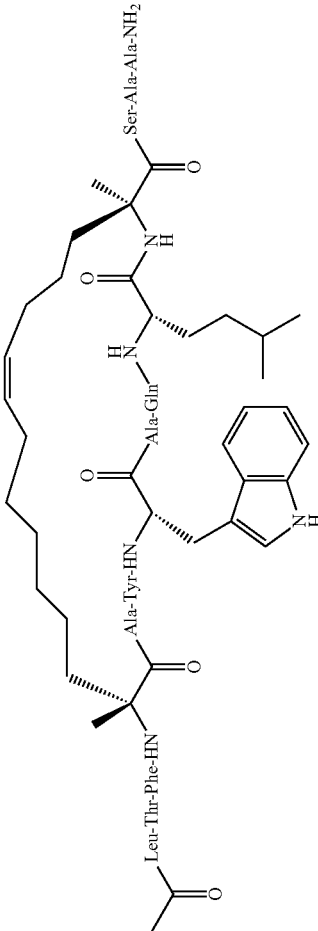
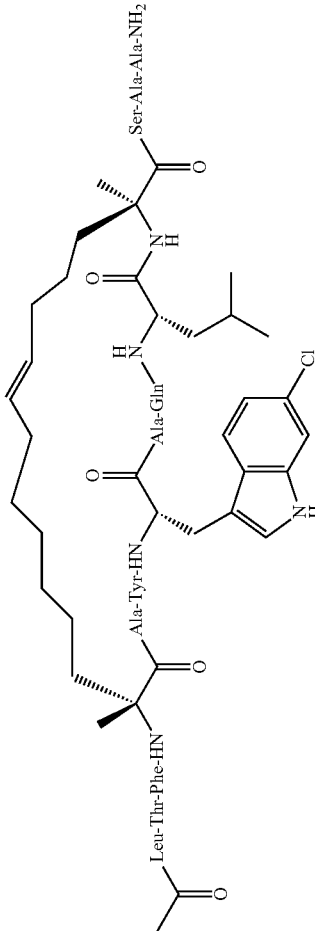
SP114 (SEQ ID NO: 123)	Structure
	 Ac-L T F \$zr8AYWAQhL\$z SAA -NH₂ Chemical Formula: C₈₃H₁₂₃N₁₇O₁₉ Exact Mass: 1687.93 Molecular Weight: 1689.00
SP99 (SEQ ID NO: 108)	 Ac-L T F \$er8AY6clWAQl\$eSAA -NH₂ Chemical Formula: C₈₄H₁₂₂ClN₁₇O₁₉ Exact Mass: 1707.88 Molecular Weight: 1709.42

TABLE 1c-continued

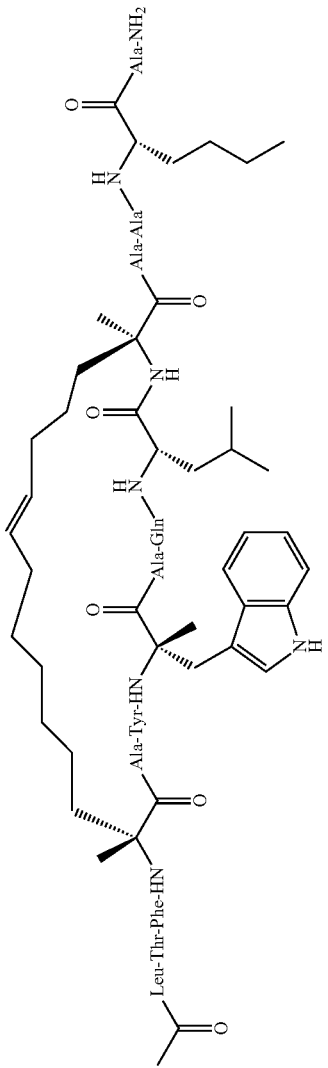
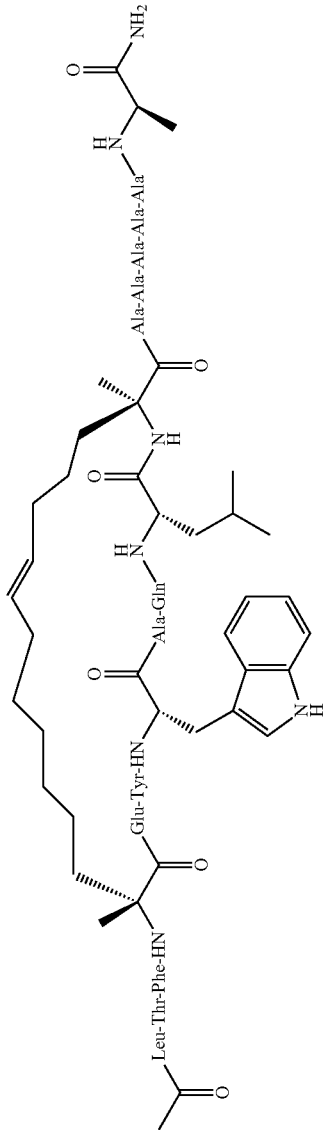
Structure	
SP388 (SEQ ID NO: 397)  Ac-L T F Ser8AYAmwAQLSeAA Nle A-NH2 Chemical Formula: C₉₇H₁₃₆N₁₈O₁₉ Exact Mass: 1785.02 Molecular Weight: 1786.16	SP331 (SEQ ID NO: 340)  Ac-L T F Ser8EYWALSeAAAAA a -NH2 Chemical Formula: C₉₂H₁₄₀N₂₀O₂₃ Exact Mass: 1929.04 Molecular Weight: 1930.25

TABLE 1c-continued

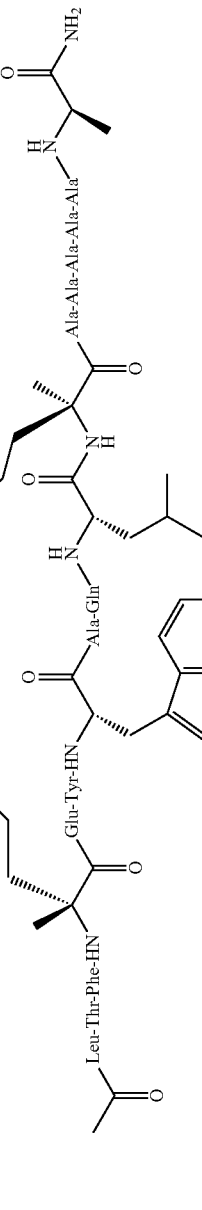
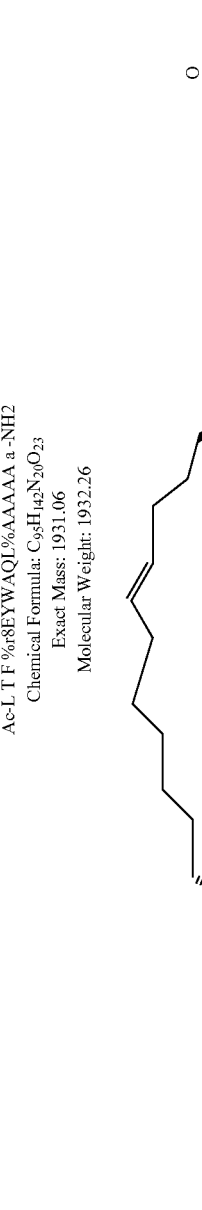
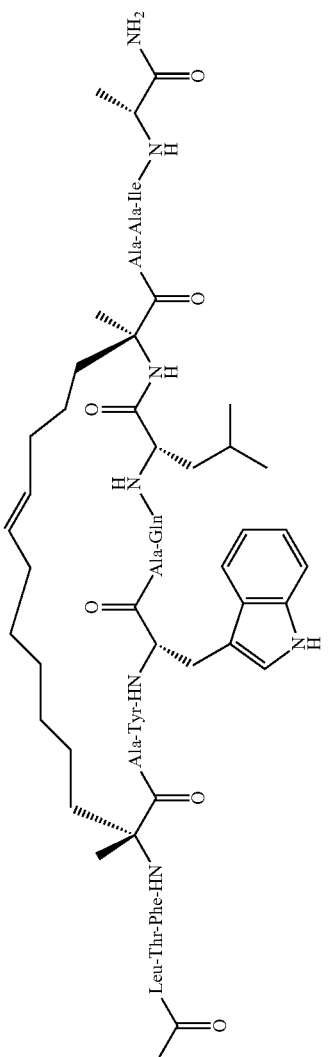
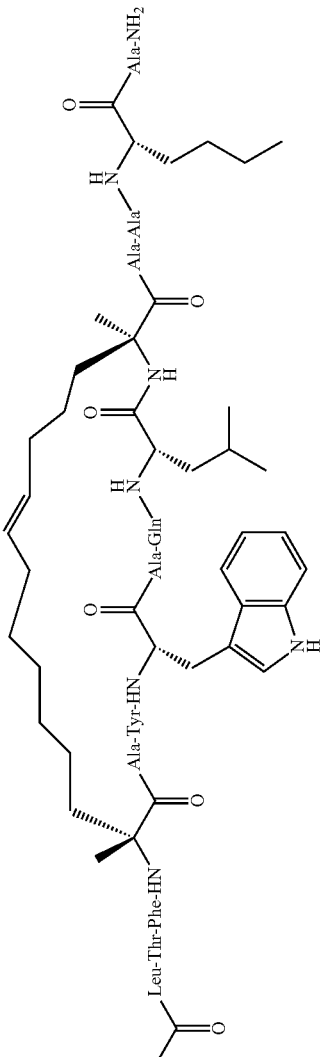
	Structure
SP445 (SEQ ID NO: 454)	 Ac-L T F %r8EYW A Q L %A A A A A a -NH2 Chemical Formula: C₉₅H₁₄₂N₂₀O₂₃ Exact Mass: 1931.06 Molecular Weight: 1932.26
SP351 (SEQ ID NO: 360)	 Ac-L T F %c r8EYWSQCb a %e A A A A A a -NH2 Chemical Formula: C₉₆H₁₄₀N₂₀O₂₄ Exact Mass: 1957.03 Molecular Weight: 1958.26

TABLE 1c-continued

Structure	
	Ac-L T F Ser8AYW AQLSeAA Ia -NH2 Chemical Formula: C₉₀H₁₃₄N₁₈O₁₉ Exact Mass: 1771.01 Molecular Weight: 1772.14
	Ac-L T F Ser8AYW AQLSeAA Nle A -NH2 Chemical Formula: C₉₀H₁₃₄N₁₈O₁₉ Exact Mass: 1771.01 Molecular Weight: 1772.14

SP71
(SEQ
ID
NO:
80)

SP69
(SEQ
ID
NO:
78)

TABLE 1c-continued

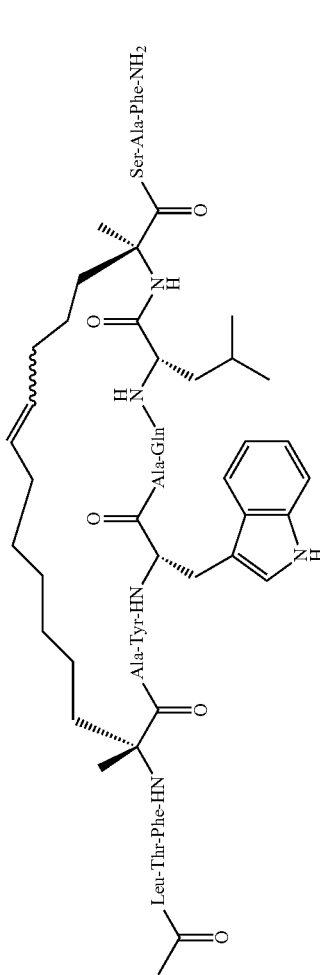
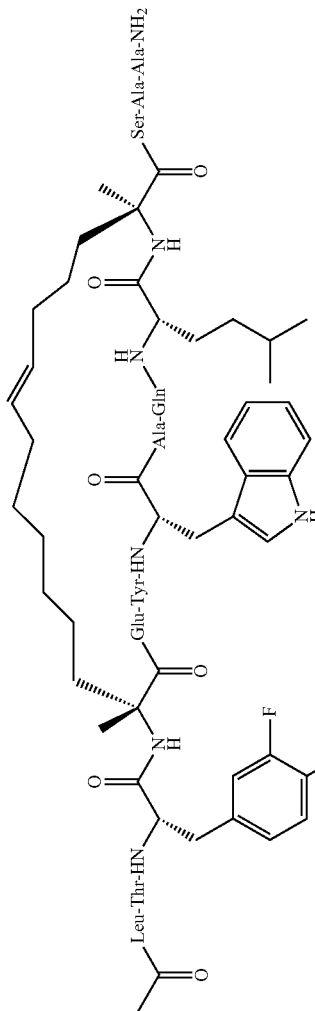
Structure	
SP7 (SEQ ID NO: 16)	 Ac-L T F \$r8AYWAQL\$ SA F -NH2 Chemical Formula: C₉₀H₁₂₇N₁₇O₁₉ Exact Mass: 1749.95 Molecular Weight: 1751.07
SP160 (SEQ ID NO: 169)	 Ac-L T F34F2Ser8EYW AQL\$eSAA -NH2 Chemical Formula: C₉₇H₁₂₅F₂N₁₇O₂₁ Exact Mass: 1781.92 Molecular Weight: 1783.02

TABLE 1c-continued

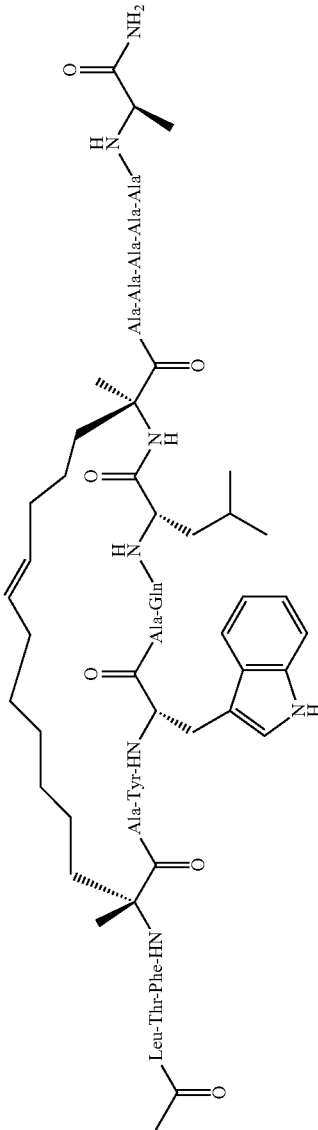
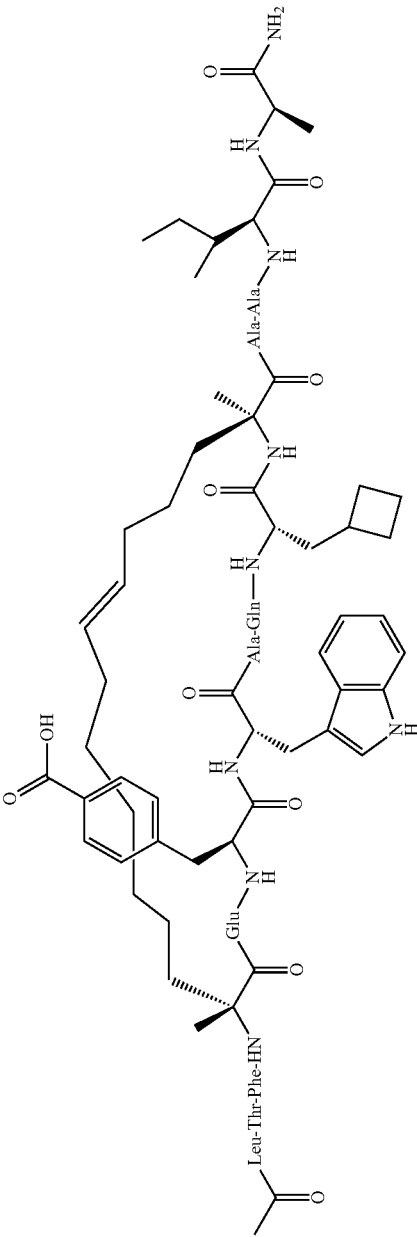
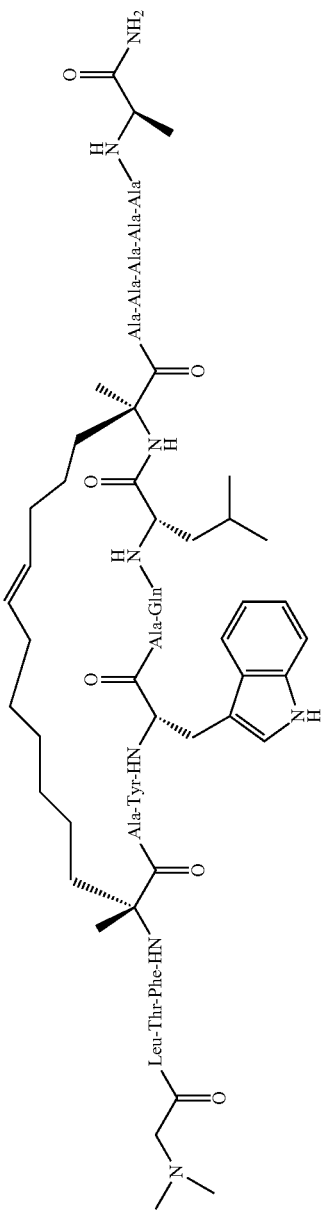
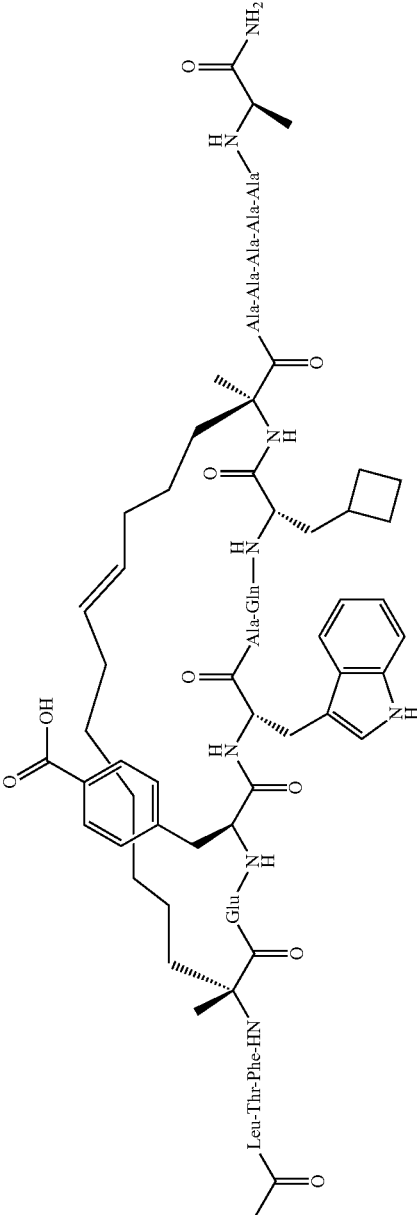
Structure	
 SP315 (SEQ ID NO: 324) Ac-L T F Ser8AYW AQLSeAAAAA a -NH2 Chemical Formula: C₉₃H₁₃₈N₂₀O₂₁ Exact Mass: 1871.03 Molecular Weight: 1872.21	 SP249 (SEQ ID NO: 258) Ac-L T F Ser8EF4cooh WAQCbaSe AA-I-a -NH2 Chemical Formula: C₉₄H₁₃₆N₁₈O₂₂ Exact Mass: 1869.01 Molecular Weight: 1870.19

TABLE 1c-continued

Structure	
	Dmaac- L TF Ser8AYWAOQLSeAAAAA a-NH2 Chemical Formula: $C_{93}H_{143}N_2O_{21}$ Exact Mass: 1914.08 Molecular Weight: 1915.28
	Ac-L TF Ser8EF4cooh WAQCbaSe AAAAA a-NH2 Chemical Formula: $C_{97}H_{140}N_2O_{24}$ Exact Mass: 1969.03 Molecular Weight: 1970.27

SP437
(SEQ
ID
NO:
446)

SP349
(SEQ
ID
NO:
358)

101

102

TABLE 1c-continued

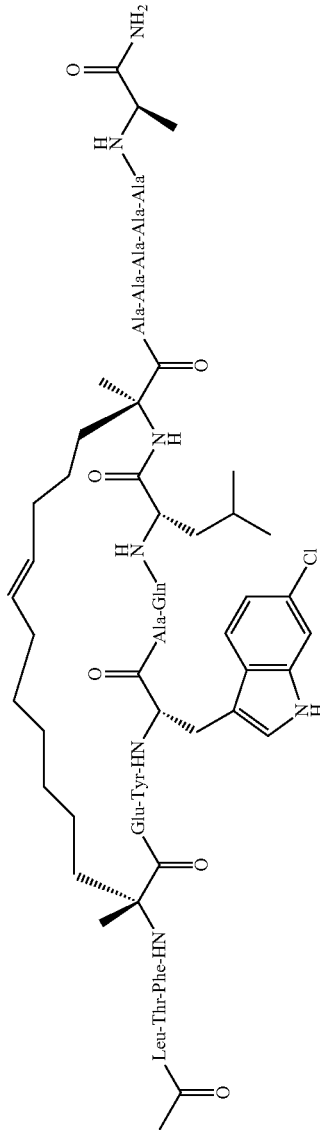
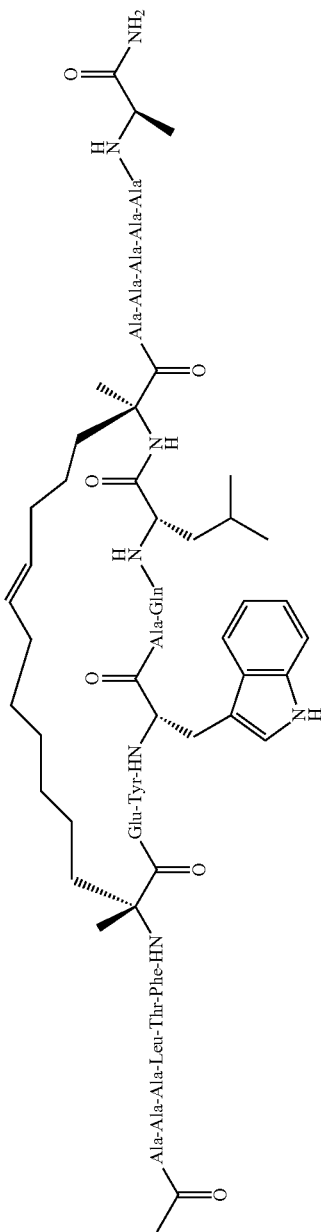
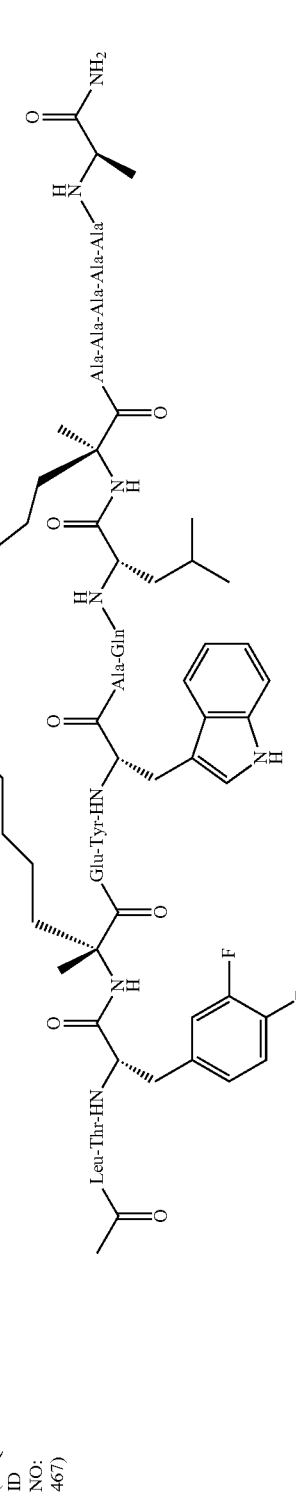
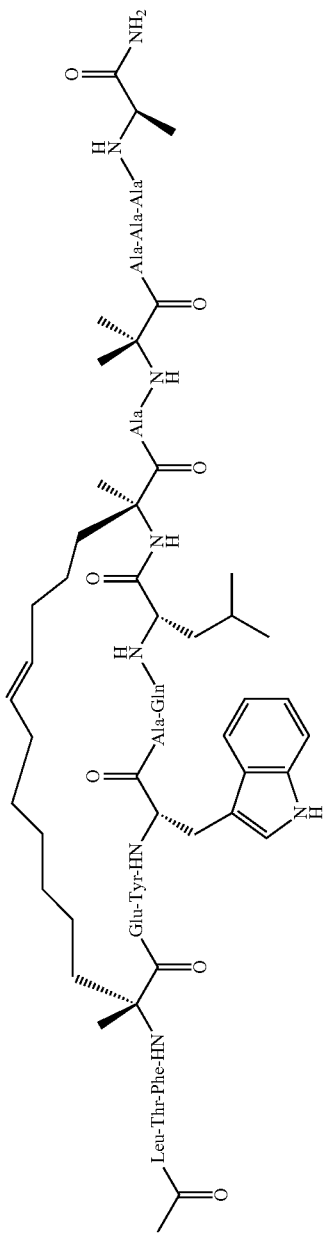
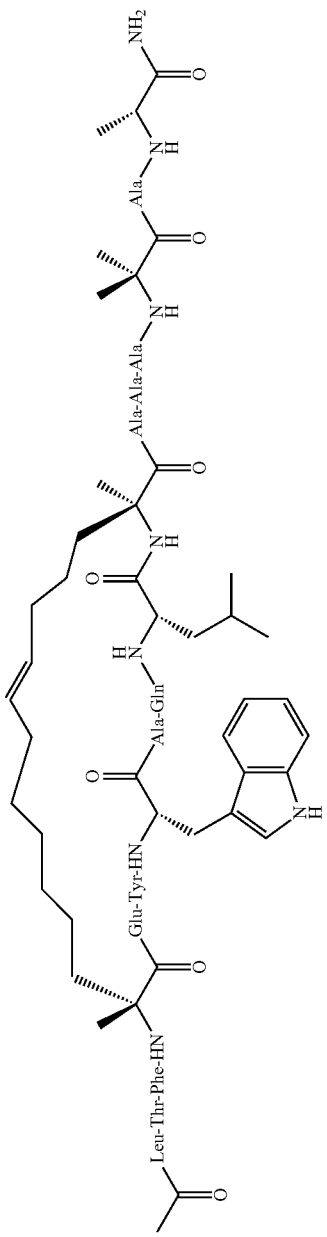
Structure	
SP555 (SEQ ID NO: 464)  Ac-L T F Ser8EY6clWAQLSe AAAAA a -NH2 Chemical Formula: C₉₅H₁₃₉ClN₂₀O₂₃ Exact Mass: 1963.00 Molecular Weight: 1964.69	
SP557 (SEQ ID NO: 466)  Ac-AAA L T F Ser8EYWAQLSe AAAAA a -NH2 Chemical Formula: C₁₀₄H₁₅₅N₂₃O₂₆ Exact Mass: 2142.15 Molecular Weight: 2143.48	

TABLE 1c-continued

SP558 (SEQ ID NO: 467)	Structure
	
	Ac-L T F34F2Ser8EYWAQLSe AAAAA a-NH2
	Chemical Formula: C ₉₅ H ₁₃₈ F ₂ N ₂₀ O ₂₃
	Exact Mass: 1965.02
	Molecular Weight: 1966.23

5-EAM- Ba L T F Ser8EYWAQCba \$e SAA -NH2

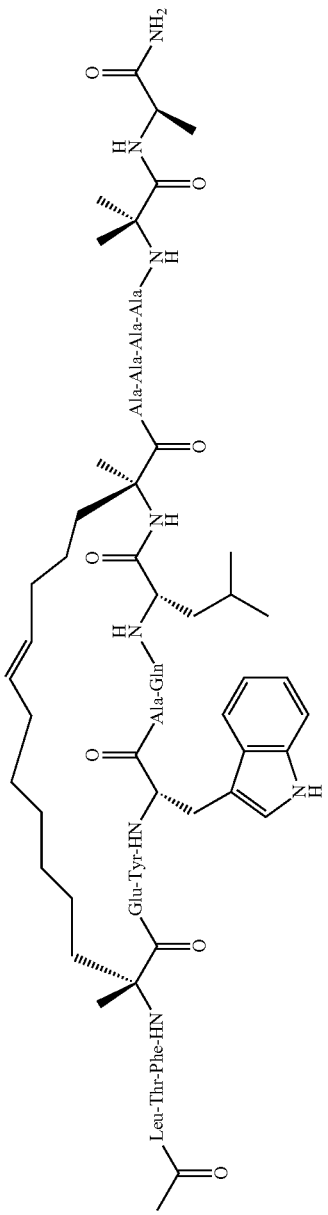
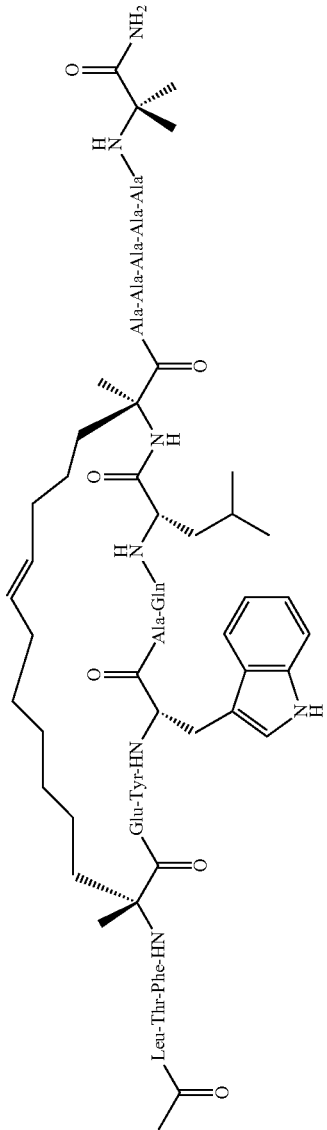
TABLE 1c-continued

Structure	
	Ac-L T F \$er8EYW\$AQL\$e A Aib AAA a -NH2 Chemical Formula: C₉₆H₁₄₂N₂₆O₂₃ Exact Mass: 1943.06 Molecular Weight: 1944.27
	Ac-L T F \$er8EYW\$AQL\$e A Aib AAA A a -NH2 Chemical Formula: C₉₆H₁₄₂N₂₆O₂₃ Exact Mass: 1943.06 Molecular Weight: 1944.27

SP562
(SEQ
ID
NO:
471)

SP564
(SEQ
ID
NO:
473)

TABLE 1c-continued

	Structure
SP566 (SEQ ID NO: 475)	
SP567 (SEQ ID NO: 476)	

Ac-L T F Ser8YWAQLSeAAAAA Aib -NH2
Chemical Formula: C₉₉H₁₄₂N₂₀O₂₃
Exact Mass: 1943.06
Molecular Weight: 1944.27

Structure

Ac-L T F Ser8EYWAQLSeAAAA a -NH2
Chemical Formula: C₉₅H₁₄₀N₂₀O₂₃
Exact Mass: 1929.04
Molecular Weight: 1930.25

Ac-L T F \$e8EYWAQL\$e AAAAAA -NH2
Chemical Formula: C₉₅H₁₄₀N₂₀O₂₃
Exact Mass: 1929.04
Molecular Weight: 1930.25

111

112

TABLE 1c-continued

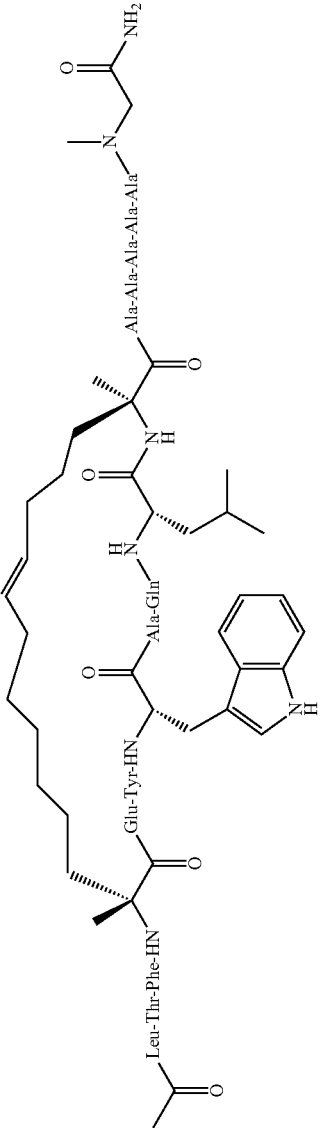
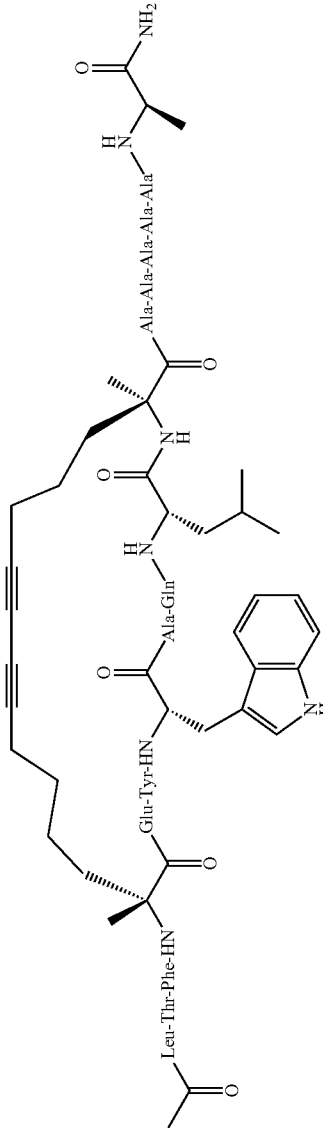
SP578 (SEQ ID NO: 487)	Structure
	 Ac-LTF\$rd6EYWAQL\$da5AAAAa-NH2 Chemical Formula: C₉₃H₁₄₀N₂₀O₂₃ Exact Mass: 1929.04 Molecular Weight: 1930.25
SP664 (SEQ ID NO: 572)	 Ac-LTF\$rd6EYWAQL\$da5AAAAa-NH2 Chemical Formula: C₉₃H₁₃₄N₂₀O₂₃ Exact Mass: 1922.99 Molecular Weight: 1924.20

TABLE 1c-continued

Structure
SP662 (SEQ ID NO: 570) Ac-L T F Srd6EYWAQL Sda5 AAAAA a-NH2 Chemical Formula: $C_{93}H_{134}N_{20}O_{23}$ Exact Mass: 1922.99 Molecular Weight: 1924.20
(SEQ ID NO: 1500) Chemical Formula: $C_{96}H_{136}N_{20}O_{23}$ Exact Mass: 1937.01 Molecular Weight: 1938.23

In some embodiments, peptidomimetic macrocycles exclude peptidomimetic macrocycles shown in Table 2a:

TABLE 2a

Number	Sequence	SEQ ID NO:
1	L\$5QETFS\$8WKLLPEN	693
2	LSQ\$5TFSDLW\$8LLPEN	694
3	LSQE\$5FSDLWK\$8LPEN	695
4	LSQET\$5 SDLWKL\$8PEN	696
5	LSQETF\$5DLWKLL\$8EN	697
6	LXQETFS\$5DLWKLLP\$8N	698
7	LSQETFS\$5WKLLPE\$8	699
8	LSQQTFS\$5DLWKLL\$8EN	700
9	LSQETF\$5DLWKLL\$8QN	701
10	LSQQTFS\$5DLWKLL\$8QN	702
11	LSQETF\$5NLWKLL\$8QN	703
12	LSQQTFS\$5NLWKLL\$8QN	704
13	LSQQTFS\$5NLWRL\$8QN	705
14	QSQQTFS\$5NLWKLL\$8QN	706
15	QSQQTFS\$5NLWRL\$8QN	707
16	QSQQTAS\$5NLWRL\$8QN	708
17	L\$8QETFS\$8WKLLPEN	709
18	LSQ\$8TFSDLW\$8LPEN	710
19	LSQE\$8FSDLWK\$8LPEN	711
20	LSQET\$8SDLWKL\$8PEN	712
21	LSQETF\$8DLWKLL\$8EN	713
22	LXQETFS\$8DLWKLLP\$8N	714
23	LSQETFS\$8DLWKLLPE\$8	715
24	LSQQTFS\$8DLWKLL\$8EN	716
25	LSQETFS\$8DLWKLL\$8QN	717
26	LSQQTFS\$8DLWKLL\$8QN	718
27	LSQETFS\$8NLWKLL\$8QN	719
28	LSQQTFS\$8NLWKLL\$8QN	720
29	LSQQTFS\$8NLWRL\$8QN	721
30	QSQQTFS\$8NLWKLL\$8QN	722

TABLE 2a-continued

Number	Sequence	SEQ ID NO:
31	QSQQTFS\$8NLWRL\$8QN	723
32	QSQQTAS\$8NLWRL\$8QN	724
33	QSQQTFS\$8NLWRK\$8QN	725
34	QQTFS\$8DLWRL\$8EN	726
35	QQTFS\$8DLWRL\$8S	727
36	LSQQTFS\$DLW\$8LL	728
37	QQTFS\$DLW\$8LL	729
38	QQTAS\$8DLWRL\$8EN	730
39	QSQQTFS\$5NLWRL\$8QN (dihydroxylated olefin)	731
40	QSQQTAS\$5NLWRL\$8QN (dihydroxylated olefin)	732
41	QSQQTFS\$8DLWRL\$8QN	733
42	QTF\$8NLWRL\$8S	734
43	QSQQTFS\$NLW\$8LLPQN	735
44	QSSQTF\$NLWRL\$8PQN	736
45	\$TF\$8DLW\$8LL	737
46	ETFS\$DLW\$8LL	738
47	QTF\$NLW\$8LL	739
48	\$SQE\$F\$NLWKLL	740

In Table 2a, X represents S or any amino acid. Peptides shown can comprise an N-terminal capping group such as acetyl or an additional linker such as beta-alanine between the capping group and the start of the peptide sequence.

In some embodiments, peptidomimetic macrocycles do not comprise a peptidomimetic macrocycle structure as shown in Table 2a.

In other embodiments, peptidomimetic macrocycles exclude peptidomimetic macrocycles shown in Table 2b:

TABLE 2b

Number	Sequence	SEQ ID NO:	Exact Mass	M + 2	Observed mass (m/e)
1	Ac-LSQETFS\$8DLWKLL\$8EN-NH2	741	2068.13	1035.07	1035.36
2	Ac-LSQETFS\$8NLWKLL\$8QN-NH2	742	2066.16	1034.08	1034.31
3	Ac-LSQQTFS\$8NLWRL\$8QN-NH2	743	2093.18	1047.59	1047.73
4	Ac-QSQQTFS\$8NLWKLL\$8QN-NH2	744	2080.15	1041.08	1041.31
5	Ac-QSQQTFS\$8NLWRL\$8QN-NH2	745	2108.15	1055.08	1055.32
6	Ac-QSQQTAS\$8NLWRL\$8QN-NH2	746	2032.12	1017.06	1017.24
7	Ac-QAibQQTFS\$8NLWRL\$8QN-NH2	747	2106.17	1054.09	1054.34
8	Ac-QSQQTFS\$NLWRL\$8PQN-NH2	748	2000.02	1001.01	1001.26
9	Ac-QSQQTFS\$8NLWRL\$8QN-NH2	749	2136.18	1069.09	1069.37
10	Ac-QSQQAibTF\$8NLWRL\$8QN-NH2	750	2065.15	1033.58	1033.71
11	Ac-QSQQTFS\$8NLWRL\$8AN-NH2	751	2051.13	1026.57	1026.70
12	Ac-ASQQTFS\$8NLWRL\$8QN-NH2	752	2051.13	1026.57	1026.90
13	Ac-QSQQTFS\$8ALWRL\$8QN-NH2	753	2065.15	1033.58	1033.41
14	Ac-QSQETFS\$8NLWRL\$8QN-NH2	754	2109.14	1055.57	1055.70
15	Ac-RSQQTFS\$8NLWRL\$8QN-NH2	755	2136.20	1069.10	1069.17
16	Ac-RSQQTFS\$8NLWRL\$8EN-NH2	756	2137.18	1069.59	1069.75
17	Ac-LSQETFS\$DLWKLLPEN-NH2	757	1959.99	981.00	981.24
18	Ac-QSQ\$TF\$8DLWRL\$8PQN-NH2	758	2008.09	1005.05	1004.97
19	Ac-QSQQ\$F\$8DLWRL\$8PQN-NH2	759	2036.06	1019.03	1018.86
20	Ac-QSQQT\$8NL\$8RL\$8PQN-NH2	760	1917.04	959.52	959.32
21	Ac-QSQQTFS\$NLW\$8LLPQN-NH2	761	2007.06	1004.53	1004.97
22	Ac-RTQATFS\$8NQWAibANle\$8TNAibTR-NH2	762	2310.26	1156.13	1156.52
23	Ac-QSQQTFS\$8NLWRL\$8RN-NH2	763	2136.20	1069.10	1068.94
24	Ac-QSQRTFS\$8NLWRL\$8QN-NH2	764	2136.20	1069.10	1068.94
25	Ac-QSQQTFS\$8NNle\$8WRL\$8QN-NH2	765	2108.15	1055.08	1055.44
26	Ac-QSQQTFS\$8NLWRL\$8Nle\$8QN-NH2	766	2108.15	1055.08	1055.84
27	Ac-QSQQTFS\$8NLWRL\$8Nle\$8QN-NH2	767	2108.15	1055.08	1055.12
28	Ac-QSQQTY\$8NLWRL\$8QN-NH2	768	2124.15	1063.08	1062.92
29	Ac-RAibQQTFS\$8NLWRL\$8QN-NH2	769	2134.22	1068.11	1068.65
30	Ac-MPRFMDYWEGLN-NH2	770	1598.70	800.35	800.45
31	Ac-RSQQRF\$8NLWRL\$8QN-NH2	771	2191.25	1096.63	1096.83
32	Ac-QSQQRF\$8NLWRL\$8QN-NH2	772	2163.21	1082.61	1082.87
33	Ac-RAibQQRF\$8NLWRL\$8QN-NH2	773	2189.27	1095.64	1096.37
34	Ac-RSQQRF\$8NFWRLL\$8QN-NH2	774	2225.23	1113.62	1114.37
35	Ac-RSQQRF\$8NYWRL\$8QN-NH2	775	2241.23	1121.62	1122.37
36	Ac-RSQQTFS\$8NLWQL\$8QN-NH2	776	2108.15	1055.08	1055.29
37	Ac-QSQQTFS\$8NLWQAmlL\$8QN-NH2	777	2094.13	1048.07	1048.32

TABLE 2b-continued

Number	Sequence	SEQ ID NO:	Exact Mass	M + 2	Observed mass (m/e)
38	Ac-QSQQTF\$r8NAmIWRLL\$QN-NH2	778	2122.17	1062.09	1062.35
39	Ac-NlePRF\$r8DYWEGL\$QN-NH2	779	1869.98	935.99	936.20
40	Ac-NlePRF\$r8NYWRLL\$QN-NH2	780	1952.12	977.06	977.35
41	Ac-RF\$r8NLWRLL\$Q-NH2	781	1577.96	789.98	790.18
42	Ac-QSQQTF\$r8N2fWRLL\$QN-NH2	782	2160.13	1081.07	1081.40
43	Ac-QSQQTF\$r8N3fWRLL\$QN-NH2	783	2160.13	1081.07	1081.34
44	Ac-QSQQTF#r8NLWRLL\$QN-NH2	784	2080.12	1041.06	1041.34
45	Ac-RSQQTAS\$r8NLWRLL\$QN-NH2	785	2060.16	1031.08	1031.38
46	Ac-QSQQTF%r8NLWRLL\$QN-NH2	786	2110.17	1056.09	1056.55
47	HepQSQ\$TF\$NLWRLLPQN-NH2	787	2051.10	1026.55	1026.82
48	HepQSQ\$TF\$r8NLWRLL\$QN-NH2	788	2159.23	1080.62	1080.89
49	Ac-QSQQTF\$r8NL6clWRLL\$QN-NH2	789	2142.11	1072.06	1072.35
50	Ac-QSQQTF\$r8NLMe6clWRLL\$QN-NH2	790	2156.13	1079.07	1079.27
51	Ac-LTFEHYWAQLTS-NH2	791	1535.74	768.87	768.91
52	Ac-LTF\$HYW\$QLTS-NH2	792	1585.83	793.92	794.17
53	Ac-LTFE\$YWA\$LTS-NH2	793	1520.79	761.40	761.67
54	Ac-LTF\$zr8HYWAQL\$zS-NH2	794	1597.87	799.94	800.06
55	Ac-LTF\$r8HYWRQL\$S-NH2	795	1682.93	842.47	842.72
56	Ac-QSQ\$TF\$NLWRLL\$8QN-NH2	796	2145.21	1073.61	1073.90
57	Ac-QSQQTAS\$NLWRLLPQN-NH2	797	1923.99	963.00	963.26
58	Ac-QSQQTAS\$/r8NLWRLL\$QN-NH2	798	2060.15	1031.08	1031.24
59	Ac-ASQQTF\$/r8NLWRLL\$QN-NH2	799	2079.16	1040.58	1040.89
60	Ac-\$SQ\$F\$NLWRLLAibQN-NH2	800	2009.09	1005.55	1005.86
61	Ac-QSQ\$TF\$NLWRLLAibQN-NH2	801	2023.10	1012.55	1012.79
62	Ac-QSQ\$F\$NLWRLLAibQN-NH2	802	2024.06	1013.03	1013.31
63	Ac-QSQQTF\$NLW\$LLAibQN-NH2	803	1995.06	998.53	998.87
64	Ac-QSQQTF\$SLWR\$LLAibQN-NH2	804	2011.06	1006.53	1006.83
65	Ac-QSQQTF\$NLW\$LLAS-NH2	805	1940.02	971.01	971.29
66	Ac-\$/SQ\$/F\$NLWRLLAibQN-NH2	806	2037.12	1019.56	1019.78
67	Ac-QS\$/QTF\$/NLWRLLAibQN-NH2	807	2051.13	1026.57	1026.90
68	Ac-QSQ\$F\$NLWRLLAibQN-NH2	808	2052.09	1027.05	1027.36
69	Ac-QSQQTF\$/NLW\$/LLAibQN-NH2	809	2023.09	1012.55	1013.82
70	Ac-QSQ\$TF\$SLWRLLAibQN-NH2	810	1996.09	999.05	999.39
71	Ac-QSQ\$/TF\$/NLWRLLAibQN-NH2	811	2024.12	1013.06	1013.37
72	Ac-QS\$/QTF\$/NLWRLL\$/8QN-NH2	812	2201.27	1101.64	1102.00
73	Ac-\$r8SQQTF\$NLWRLLAibQN-NH2	813	2038.14	1020.07	1020.23
74	Ac-QSQ\$r8TF\$NLWRLLAibQN-NH2	814	1996.08	999.04	999.32
75	Ac-QSQQTF\$r8LWRLLAS-NH2	815	2024.12	1013.06	1013.37
76	Ac-QS\$r5QTF\$NLW\$LLAibQN-NH2	816	2032.12	1017.06	1017.39
77	Ac-\$/r8SQQTF\$/NLWRLLAibQN-NH2	817	2066.17	1034.09	1034.80
78	Ac-QSQ\$/r8TF\$NLW\$/LLAibQN-NH2	818	2024.11	1013.06	1014.34
79	Ac-QSQQTF\$/r8LWRLLAS/N-NH2	819	2052.15	1027.08	1027.16
80	Ac-QS\$/r5QTF\$/NLW\$/LLAibQN-NH2	820	2088.18	1045.09	1047.10
81	Ac-QSQQTF\$NLWRLLAibQN-NH2	821	1988.02	995.01	995.31
82	Hep/QSQ\$/TF\$/r8NLWRLL\$/QN-NH2	822	2215.29	1108.65	1108.93
83	Ac-ASQQTF\$/r8NLWRLL\$QN-NH2	823	2051.13	1026.57	1026.90
84	Ac-QSQQTF\$/r8NLWRLL\$/Q-NH2	824	2022.14	1012.07	1012.66
85	Ac-QSQQTF\$/r8NLWRLL\$Q-NH2	825	1994.11	998.06	998.42
86	Ac-AAARAA\$r8AARAA\$AA-NH2	826	1515.90	758.95	759.21
87	Ac-LTFEHYWAQLTSA-NH2	827	1606.78	804.39	804.59
88	Ac-LTF\$r8HYWAQL\$SA-NH2	828	1668.90	835.45	835.67
89	Ac-ASQQTF\$NLWRLLPQN-NH2	829	1943.00	972.50	973.27
90	Ac-QSQ\$TF\$NLW\$/5LLAibQN-NH2	830	2032.12	1017.06	1017.30
91	Ac-QSQQTFaibNLWRLLAibQN-NH2	831	1986.04	994.02	994.19
92	Ac-QSQQTFNleNLWRLLNleQN-NH2	832	2042.11	1022.06	1022.23
93	Ac-QSQQTF\$/r8NLWRLLAibQN-NH2	833	2082.14	1042.07	1042.23
94	Ac-QSQQTF\$/r8NLWRLLNleQN-NH2	834	2110.17	1056.09	1056.29
95	Ac-QSQQTFaibNLWRLL\$/QN-NH2	835	2040.09	1021.05	1021.25
96	Ac-QSQQTFNleNLWRLL\$/QN-NH2	836	2068.12	1035.06	1035.31
97	Ac-QSQQTF%r8NL6clWRNleL\$QN-NH2	837	2144.13	1073.07	1073.32
98	Ac-QSQQTF%r8NLMe6clWRLL\$QN-NH2	838	2158.15	1080.08	1080.31
101	Ac-FNle\$YWE\$LL-NH2	839	1160.63	—	1161.70
102	Ac-F\$r8AYWELL\$A-NH2	840	1344.75	—	1345.90
103	Ac-F\$r8AYWQLL\$A-NH2	841	1343.76	—	1344.83
104	Ac-NlePRF\$r8NYWELL\$QN-NH2	842	1925.06	963.53	963.69
105	Ac-NlePRF\$r8DYWRLL\$QN-NH2	843	1953.10	977.55	977.68
106	Ac-NlePRF\$r8NYWRLL\$QN-NH2	844	1838.07	920.04	920.18
107	Ac-NlePRF\$r8NYWRLL\$-NH2	845	1710.01	856.01	856.13
108	Ac-QSQQTF\$/r8DLWRLL\$QN-NH2	846	2109.14	1055.57	1055.64
109	Ac-QSQQTF\$/r8NLWRLL\$EN-NH2	847	2109.14	1055.57	1055.70
110	Ac-QSQQTF\$/r8NLWRLL\$QD-NH2	848	2109.14	1055.57	1055.64
111	Ac-QSQQTF\$/r8NLWRLL\$S-NH2	849	1953.08	977.54	977.60
112	Ac-ESQQTF\$/r8NLWRLL\$QN-NH2	850	2109.14	1055.57	1055.70
113	Ac-LTF\$/r8NLWRNleL\$Q-NH2	851	1635.99	819.00	819.10
114	Ac-LRF\$/r8NLWRNleL\$Q-NH2	852	1691.04	846.52	846.68
115	Ac-QSQQTF\$/r8NWWRNleL\$QN-NH2	853	2181.15	1091.58	1091.64
116	Ac-QSQQTF\$/r8NLWRNleL\$Q-NH2	854	1994.11	998.06	998.07

TABLE 2b-continued

Number	Sequence	SEQ ID NO:	Exact Mass	M + 2	Observed mass (m/e)
117	Ac-QTF\$r8NLWRNleL\$QN-NH2	855	1765.00	883.50	883.59
118	Ac-NlePRF\$r8NWWRL\$QN-NH2	856	1975.13	988.57	988.75
119	Ac-NlePRF\$r8NWWRL\$A-NH2	857	1804.07	903.04	903.08
120	Ac-TSFAEYWNLLNH2	858	1467.70	734.85	734.90
121	Ac-QTF\$r8HWWSQL\$S-NH2	859	1651.85	826.93	827.12
122	Ac-FM\$YWE\$S-NH2	860	1178.58	—	1179.64
123	Ac-QTFEHWWSQLS-NH2	861	1601.76	801.88	801.94
124	Ac-QSQQTF\$r8NLAmwRLNle\$QN-NH2	862	2122.17	1062.09	1062.24
125	Ac-FMAibY6clWEAc3cL-NH2	863	1130.47	—	1131.53
126	Ac-FNle\$Y6clWE\$S-NH2	864	1194.59	—	1195.64
127	Ac-F\$zr8AY6clWEAc3cL\$z-NH2	865	1277.63	639.82	1278.71
128	Ac-F\$r8AY6clWEAc3cL\$A-NH2	866	1348.66	—	1350.72
129	Ac-NlePRF\$r8NY6clWRLL\$QN-NH2	867	1986.08	994.04	994.64
130	Ac-AF\$r8AAWALA\$A-NH2	868	1223.71	—	1224.71
131	Ac-TF\$r8AAWRLA\$Q-NH2	869	1395.80	698.90	399.04
132	Pr-TF\$r8AAWRLA\$Q-NH2	870	1409.82	705.91	706.04
133	Ac-QSQQTF%r8NLWRNleL%QN-NH2	871	2110.17	1056.09	1056.22
134	Ac-LTF%r8HYWAQL%SA-NH2	872	1670.92	836.46	836.58
135	Ac-NlePRF%r8NYWRLL%QN-NH2	873	1954.13	978.07	978.19
136	Ac-NlePRF%r8NY6clWRLL%QN-NH2	874	1988.09	995.05	995.68
137	Ac-LTF%r8HY6clWAQL%S-NH2	875	1633.84	817.92	817.93
138	Ac-QS%QTF%StNLWRLL%8QN-NH2	876	2149.24	1075.62	1075.65
139	Ac-LTF%r8HY6clWRQL%S-NH2	877	1718.91	860.46	860.54
140	Ac-QSQQTF%r8NL6clWRLL%QN-NH2	878	2144.13	1073.07	1073.64
141	Ac-%r8SQQTF%LWRLLAibQN-NH2	879	2040.15	1021.08	1021.13
142	Ac-LTF%r8HYWAQL%S-NH2	880	1599.88	800.94	801.09
143	Ac-TSF%r8QYWNLL%P-NH2	881	1602.88	802.44	802.58
147	Ac-LTFEHYWAQL\$S-NH2	882	1535.74	768.87	769.5
152	Ac-F\$er8AY6clWEAc3cL\$e-NH2	883	1277.63	639.82	1278.71
153	Ac-AF\$r8AAWALA\$A-NH2	884	1277.63	639.82	1277.84
154	Ac-TF\$r8AAWRLA\$Q-NH2	885	1395.80	698.90	699.04
155	Pr-TF\$r8AAWRLA\$Q-NH2	886	1409.82	705.91	706.04
156	Ac-LTF\$er8HYWAQL\$eS-NH2	887	1597.87	799.94	800.44
159	Ac-CCPGCCBaQSQQTF\$r8NLWRLL\$QN-NH2	888	2745.30	1373.65	1372.99
160	Ac-CCPGCCBaQSQQTAS\$r8NLWRLL\$QN-NH2	889	2669.27	1335.64	1336.09
161	Ac-CCPGCCBaNlePRF\$r8NYWRLL\$QN-NH2	890	2589.26	1295.63	1296.2
162	Ac-LTF\$/r8HYWAQL\$S-NH2	891	1625.90	813.95	814.18
163	Ac-F%r8HY6clWRAc3cL%-NH2	892	1372.72	687.36	687.59
164	Ac-QTF%r8HWWSQL%S-NH2	893	1653.87	827.94	827.94
165	Ac-LTA\$r8HYWRQL\$S-NH2	894	1606.90	804.45	804.66
166	Ac-Q\$r8QQTF\$NS\$WRLAibQN-NH2	895	2080.12	1041.06	1041.61
167	Ac-QSQQ\$r8FSNLWRSLAibQN-NH2	896	2066.11	1034.06	1034.58
168	Ac-F\$r8AYWEAc3cL\$A-NH2	897	1314.70	658.35	1315.88
169	Ac-F\$r8AYWEAc3cL\$S-NH2	898	1330.70	666.35	1331.87
170	Ac-F\$r8AYWEAc3cL\$Q-NH2	899	1371.72	686.86	1372.72
171	Ac-F\$r8AYWEAibL\$S-NH2	900	1332.71	667.36	1334.83
172	Ac-F\$r8AYWEAL\$S-NH2	901	1318.70	660.35	1319.73
173	Ac-F\$r8AYWEQL\$S-NH2	902	1375.72	688.86	1377.53
174	Ac-F\$r8HYWEQL\$S-NH2	903	1441.74	721.87	1443.48
175	Ac-F\$r8HYWAQL\$S-NH2	904	1383.73	692.87	1385.38
176	Ac-F\$r8HYWAAc3cL\$S-NH2	905	1338.71	670.36	1340.82
177	Ac-F\$r8HYWRAc3cL\$S-NH2	906	1423.78	712.89	713.04
178	Ac-F\$r8AYWEAc3cL\$A-NH2	907	1300.69	651.35	1302.78
179	Ac-NlePTF%r8NYWRLL%QN-NH2	908	1899.08	950.54	950.56
180	Ac-TF\$r8AAWRAL\$Q-NH2	909	1395.80	698.90	699.13
181	Ac-TSF%r8HYWAQL%S-NH2	910	1573.83	787.92	787.98
184	Ac-F%r8AY6clWEAc3cL%A-NH2	911	1350.68	676.34	676.91
185	Ac-LTF\$r8HYWAQL\$S-NH2	912	1597.87	799.94	800.07
186	Ac-LTF\$r8HYWAQNle\$S-NH2	913	1597.87	799.94	800.07
187	Ac-LTF\$r8HYWAQL\$A-NH2	914	1581.87	791.94	792.45
188	Ac-LTF\$r8HYWAQL\$Abu-NH2	915	1595.89	798.95	799.03
189	Ac-LTF\$r8HYWAbuQL\$S-NH2	916	1611.88	806.94	807.47
190	Ac-LTF\$er8AYWAQL\$eS-NH2	917	1531.84	766.92	766.96
191	Ac-LAF\$r8HYWAQL\$S-NH2	918	1567.86	784.93	785.49
192	Ac-LAF\$r8AYWAQL\$S-NH2	919	1501.83	751.92	752.01
193	Ac-LTF\$er8AYWAQL\$eA-NH2	920	1515.85	758.93	758.97
194	Ac-LAF\$r8AYWAQL\$A-NH2	921	1485.84	743.92	744.05
195	Ac-LTF\$r8NLWANleL\$Q-NH2	922	1550.92	776.46	776.61
196	Ac-LTF\$r8NLWANleL\$A-NH2	923	1493.90	747.95	1495.6
197	Ac-LTF\$r8ALWANleL\$Q-NH2	924	1507.92	754.96	755
198	Ac-LAF\$r8NLWANleL\$Q-NH2	925	1520.91	761.46	761.96
199	Ac-LAF\$r8ALWANleL\$A-NH2	926	1420.89	711.45	1421.74
200	Ac-A\$r8AYWEAc3cL\$A-NH2	927	1238.67	620.34	1239.65
201	Ac-F\$r8AYWEAc3cL\$AA-NH2	928	1385.74	693.87	1386.64
202	Ac-F\$r8AYWEAc3cL\$Abu-NH2	929	1328.72	665.36	1330.17
203	Ac-F\$r8AYWEAc3cL\$Nle-NH2	930	1356.75	679.38	1358.22
204	Ac-F\$r5AYWEAc3cL\$8A-NH2	931	1314.70	658.35	1315.51

TABLE 2b-continued

Number	Sequence	SEQ ID NO:	Exact Mass	M + 2	Observed mass (m/e)
205	Ac-F\$AYWEAc3cL\$Sr8A-NH2	932	1314.70	658.35	1315.66
206	Ac-F\$8AYWEAc3cL\$A-NH2	933	1314.70	658.35	1316.18
207	Ac-F\$8AYWEAc3cNle\$A-NH2	934	1314.70	658.35	1315.66
208	Ac-F\$8AYWEAmL\$A-NH2	935	1358.76	680.38	1360.21
209	Ac-F\$8AYWENleL\$A-NH2	936	1344.75	673.38	1345.71
210	Ac-F\$8AYWQAc3cL\$A-NH2	937	1313.72	657.86	1314.7
211	Ac-F\$8AYWAAc3cL\$A-NH2	938	1256.70	629.35	1257.56
212	Ac-F\$8AYWAbuAc3cL\$A-NH2	939	1270.71	636.36	1272.14
213	Ac-F\$8AYWNleAc3cL\$A-NH2	940	1298.74	650.37	1299.67
214	Ac-F\$8AbuYWEAc3cL\$A-NH2	941	1328.72	665.36	1329.65
215	Ac-F\$8NleYWEAc3cL\$A-NH2	942	1356.75	679.38	1358.66
216	5-FAM-BaLTFEHYWAQLTS-NH2	943	1922.82	962.41	962.87
217	5-FAM-BaLTF%r8HYWAQL%S-NH2	944	1986.96	994.48	994.97
218	Ac-LTF\$8HYWAQhL\$S-NH2	945	1611.88	806.94	807
219	Ac-LTF\$8HYWAQTle\$S-NH2	946	1597.87	799.94	799.97
220	Ac-LTF\$8HYWAQAdm\$S-NH2	947	1675.91	838.96	839.09
221	Ac-LTF\$8HYWAQhCha\$S-NH2	948	1651.91	826.96	826.98
222	Ac-LTF\$8HYWAQCha\$S-NH2	949	1637.90	819.95	820.02
223	Ac-LTF\$8HYWAQcQL\$S-NH2	950	1651.91	826.96	826.98
224	Ac-LTF\$8HYWAQcQL\$S-NH2	951	1637.90	819.95	820.02
225	Ac-LThF\$8HYWAQL\$S-NH2	952	1611.88	806.94	807
226	Ac-LTlgl\$8HYWAQL\$S-NH2	953	1625.90	813.95	812.99
227	Ac-LTF\$8HYWAQCg\$S-NH2	954	1623.88	812.94	812.99
228	Ac-LTF\$8HYWAQF\$S-NH2	955	1631.85	816.93	816.99
229	Ac-LTF\$8HYWAQlgl\$S-NH2	956	1659.88	830.94	829.94
230	Ac-LTF\$8HYWAQCba\$S-NH2	957	1609.87	805.94	805.96
231	Ac-LTF\$8HYWAQCpg\$S-NH2	958	1609.87	805.94	805.96
232	Ac-LTF\$8HhYWAQL\$S-NH2	959	1611.88	806.94	807
233	Ac-F\$8AYWEAc3chL\$A-NH2	960	1328.72	665.36	665.43
234	Ac-F\$8AYWEAc3cTle\$A-NH2	961	1314.70	658.35	1315.62
235	Ac-F\$8AYWEAc3cAdm\$A-NH2	962	1392.75	697.38	697.47
236	Ac-F\$8AYWEAc3chCha\$A-NH2	963	1368.75	685.38	685.34
237	Ac-F\$8AYWEAc3cCha\$A-NH2	964	1354.73	678.37	678.38
238	Ac-F\$8AYWEAc6cL\$A-NH2	965	1356.75	679.38	679.42
239	Ac-F\$8AYWEAc5cL\$A-NH2	966	1342.73	672.37	672.46
240	Ac-hf\$8AYWEAc3cL\$A-NH2	967	1328.72	665.36	665.43
241	Ac-lgl\$8AYWEAc3cL\$A-NH2	968	1342.73	672.37	671.5
243	Ac-F\$8AYWEAc3cF\$A-NH2	969	1348.69	675.35	675.35
244	Ac-F\$8AYWEAc3cigl\$A-NH2	970	1376.72	689.36	688.37
245	Ac-F\$8AYWEAc3cCba\$A-NH2	971	1326.70	664.35	664.47
246	Ac-F\$8AYWEAc3cCpg\$A-NH2	972	1326.70	664.35	664.39
247	Ac-F\$8AhYWEAc3cL\$A-NH2	973	1328.72	665.36	665.43
248	Ac-F\$8AYWEAc3cL\$Q-NH2	974	1371.72	686.86	1372.87
249	Ac-F\$8AYWEAibL\$A-NH2	975	1316.72	659.36	1318.18
250	Ac-F\$8AYWEAL\$A-NH2	976	1302.70	652.35	1303.75
251	Ac-LAF\$8AYWAAL\$A-NH2	977	1428.82	715.41	715.49
252	Ac-LTF\$8HYWAAc3cL\$S-NH2	978	1552.84	777.42	777.5
253	Ac-NleTF\$8HYWAQL\$S-NH2	979	1597.87	799.94	800.04
254	Ac-VTF\$8HYWAQL\$S-NH2	980	1583.85	792.93	793.04
255	Ac-FTF\$8HYWAQL\$S-NH2	981	1631.85	816.93	817.02
256	Ac-WTF\$8HYWAQL\$S-NH2	982	1670.86	836.43	836.85
257	Ac-RTF\$8HYWAQL\$S-NH2	983	1640.88	821.44	821.9
258	Ac-KTF\$8HYWAQL\$S-NH2	984	1612.88	807.44	807.91
259	Ac-LNleF\$8HYWAQL\$S-NH2	985	1609.90	805.95	806.43
260	Ac-LVF\$8HYWAQL\$S-NH2	986	1595.89	798.95	798.93
261	Ac-LFF\$8HYWAQL\$S-NH2	987	1643.89	822.95	823.38
262	Ac-LWF\$8HYWAQL\$S-NH2	988	1682.90	842.45	842.55
263	Ac-LRF\$8HYWAQL\$S-NH2	989	1652.92	827.46	827.52
264	Ac-LKF\$8HYWAQL\$S-NH2	990	1624.91	813.46	813.51
265	Ac-LTF\$8NleYWAQL\$S-NH2	991	1573.89	787.95	788.05
266	Ac-LTF\$8VYWAQL\$S-NH2	992	1559.88	780.94	780.98
267	Ac-LTF\$8FYWAQL\$S-NH2	993	1607.88	804.94	805.32
268	Ac-LTF\$8WYWAQL\$S-NH2	994	1646.89	824.45	824.86
269	Ac-LTF\$8RYWAQL\$S-NH2	995	1616.91	809.46	809.51
270	Ac-LTF\$8KYWAQL\$S-NH2	996	1588.90	795.45	795.48
271	Ac-LTF\$8HNleYWAQL\$S-NH2	997	1547.89	774.95	774.98
272	Ac-LTF\$8HVYWAQL\$S-NH2	998	1533.87	767.94	767.95
273	Ac-LTF\$8HFWAQL\$S-NH2	999	1581.87	791.94	792.3
274	Ac-LTF\$8HWWAQL\$S-NH2	1000	1620.88	811.44	811.54
275	Ac-LTF\$8HRWAQL\$S-NH2	1001	1590.90	796.45	796.52
276	Ac-LTF\$8HKWAQL\$S-NH2	1002	1562.90	782.45	782.53
277	Ac-LTF\$8HYWNleQL\$S-NH2	1003	1639.91	820.96	820.98
278	Ac-LTF\$8HYWVQL\$S-NH2	1004	1625.90	813.95	814.03
279	Ac-LTF\$8HYWFQL\$S-NH2	1005	1673.90	837.95	838.03
280	Ac-LTF\$8HYWWQL\$S-NH2	1006	1712.91	857.46	857.5
281	Ac-LTF\$8HYWKQL\$S-NH2	1007	1654.92	828.46	828.49
282	Ac-LTF\$8HYWANleL\$S-NH2	1008	1582.89	792.45	792.52

TABLE 2b-continued

Number	Sequence	SEQ ID NO:	Exact Mass	M + 2	Observed mass (m/e)
283	Ac-LTF\$r8HYWAVL\$S-NH2	1009	1568.88	785.44	785.49
284	Ac-LTF\$r8HYWAVL\$S-NH2	1010	1616.88	809.44	809.47
285	Ac-LTF\$r8HYWAWL\$S-NH2	1011	1655.89	828.95	829
286	Ac-LTF\$r8HYWARL\$S-NH2	1012	1625.91	813.96	813.98
287	Ac-LTF\$r8HYWAQL\$Nle-NH2	1013	1623.92	812.96	813.39
288	Ac-LTF\$r8HYWAQL\$V-NH2	1014	1609.90	805.95	805.99
289	Ac-LTF\$r8HYWAQL\$F-NH2	1015	1657.90	829.95	830.26
290	Ac-LTF\$r8HYWAQL\$W-NH2	1016	1696.91	849.46	849.5
291	Ac-LTF\$r8HYWAQL\$R-NH2	1017	1666.94	834.47	834.56
292	Ac-LTF\$r8HYWAQL\$K-NH2	1018	1638.93	820.47	820.49
293	Ac-QS\$r8QQTF\$NSWRLLAibQN-NH2	1019	2080.12	1041.06	1041.54
294	Ac-QSQ\$Q\$r8FSNLWRSLAibQN-NH2	1020	2066.11	1034.06	1034.58
295	Ac-LT2Pal\$r8HYWAQL\$S-NH2	1021	1598.86	800.43	800.49
296	Ac-LT3Pal\$r8HYWAQL\$S-NH2	1022	1598.86	800.43	800.49
297	Ac-LT4Pal\$r8HYWAQL\$S-NH2	1023	1598.86	800.43	800.49
298	Ac-LTF2CF3\$r8HYWAQL\$S-NH2	1024	1665.85	833.93	834.01
299	Ac-LTF2CN\$r8HYWAQL\$S-NH2	1025	1622.86	812.43	812.47
300	Ac-LTF2Me\$r8HYWAQL\$S-NH2	1026	1611.88	806.94	807
301	Ac-LTF3CI\$r8HYWAQL\$S-NH2	1027	1631.83	816.92	816.99
302	Ac-LTF4CF3\$r8HYWAQL\$S-NH2	1028	1665.85	833.93	833.94
303	Ac-LTF4Bu\$r8HYWAQL\$S-NH2	1029	1653.93	827.97	828.02
304	Ac-LTF5F\$r8HYWAQL\$S-NH2	1030	1687.82	844.91	844.96
305	Ac-LTF\$r8HY3BthAAQL\$S-NH2	1031	1614.83	808.42	808.48
306	Ac-LTF2Br\$r8HYWAQL\$S-NH2	1032	1675.78	838.89	838.97
307	Ac-LTF4Br\$r8HYWAQL\$S-NH2	1033	1675.78	838.89	839.86
308	Ac-LTF2CI\$r8HYWAQL\$S-NH2	1034	1631.83	816.92	816.99
309	Ac-LTF4CI\$r8HYWAQL\$S-NH2	1035	1631.83	816.92	817.36
310	Ac-LTF3CN\$r8HYWAQL\$S-NH2	1036	1622.86	812.43	812.47
311	Ac-LTF4CN\$r8HYWAQL\$S-NH2	1037	1622.86	812.43	812.47
312	Ac-LTF34CI2\$r8HYWAQL\$S-NH2	1038	1665.79	833.90	833.94
313	Ac-LTF34F2\$r8HYWAQL\$S-NH2	1039	1633.85	817.93	817.95
314	Ac-LTF35F2\$r8HYWAQL\$S-NH2	1040	1633.85	817.93	817.95
315	Ac-LTDip\$r8HYWAQL\$S-NH2	1041	1673.90	837.95	838.01
316	Ac-LTF2F\$r8HYWAQL\$S-NH2	1042	1615.86	808.93	809
317	Ac-LTF3F\$r8HYWAQL\$S-NH2	1043	1615.86	808.93	809
318	Ac-LTF4F\$r8HYWAQL\$S-NH2	1044	1615.86	808.93	809
319	Ac-LTF4I\$r8HYWAQL\$S-NH2	1045	1723.76	862.88	862.94
320	Ac-LTF3Me\$r8HYWAQL\$S-NH2	1046	1611.88	806.94	807.07
321	Ac-LTF4Me\$r8HYWAQL\$S-NH2	1047	1611.88	806.94	807
322	Ac-LT1NaI\$r8HYWAQL\$S-NH2	1048	1647.88	824.94	824.98
323	Ac-LT2NaI\$r8HYWAQL\$S-NH2	1049	1647.88	824.94	825.06
324	Ac-LTF3CF3\$r8HYWAQL\$S-NH2	1050	1665.85	833.93	834.01
325	Ac-LTF4NO2\$r8HYWAQL\$S-NH2	1051	1642.85	822.43	822.46
326	Ac-LTF3NO2\$r8HYWAQL\$S-NH2	1052	1642.85	822.43	822.46
327	Ac-LTF\$r82ThiYWAQL\$S-NH2	1053	1613.83	807.92	807.96
328	Ac-LTF\$r8HBipWAQL\$S-NH2	1054	1657.90	829.95	830.01
329	Ac-LTF\$r8HF4tBuWAQL\$S-NH2	1055	1637.93	819.97	820.02
330	Ac-LTF\$r8HF4CF3WAQL\$S-NH2	1056	1649.86	825.93	826.02
331	Ac-LTF\$r8HF4CIWAQL\$S-NH2	1057	1615.83	808.92	809.37
332	Ac-LTF\$r8HF4MeWAQL\$S-NH2	1058	1595.89	798.95	799.01
333	Ac-LTF\$r8HF4BrWAQL\$S-NH2	1059	1659.78	830.89	830.98
334	Ac-LTF\$r8HF4CNWAQL\$S-NH2	1060	1606.87	804.44	804.56
335	Ac-LTF\$r8HF4NO2WAQL\$S-NH2	1061	1626.86	814.43	814.55
336	Ac-LTF\$r8H1NaIWAQL\$S-NH2	1062	1631.89	816.95	817.06
337	Ac-LTF\$r8H2NaIWAQL\$S-NH2	1063	1631.89	816.95	816.99
338	Ac-LTF\$r8HWAQL\$S-NH2	1064	1434.80	718.40	718.49
339	Ac-LTF\$r8HY1NaIWAQL\$S-NH2	1065	1608.87	805.44	805.52
340	Ac-LTF\$r8HY2NaIWAQL\$S-NH2	1066	1608.87	805.44	805.52
341	Ac-LTF\$r8HYWAQL\$S-NH2	1067	1597.87	799.94	800.07
342	Ac-LTF\$r8HYWAQNle\$S-NH2	1068	1597.87	799.94	800.44
343	Ac-LTFSer8HYWAQL\$SeA-NH2	1069	1581.87	791.94	791.98
344	Ac-LTF\$r8HYWAQL\$Abu-NH2	1070	1595.89	798.95	799.03
345	Ac-LTF\$r8HYWAuQL\$S-NH2	1071	1611.88	806.94	804.47
346	Ac-LAF\$r8HYWAQL\$S-NH2	1072	1567.86	784.93	785.49
347	Ac-LTF\$r8NLWANleL\$Q-NH2	1073	1550.92	776.46	777.5
348	Ac-LTF\$r8ALWANleL\$Q-NH2	1074	1507.92	754.96	755.52
349	Ac-LAF\$r8NLWANleL\$Q-NH2	1075	1520.91	761.46	762.48
350	Ac-FS\$r8AYWAAc3eL\$A-NH2	1076	1256.70	629.35	1257.56
351	Ac-LTF\$r8AYWAAL\$S-NH2	1077	1474.82	738.41	738.55
352	Ac-LVF\$r8AYWAQL\$S-NH2	1078	1529.87	765.94	766
353	Ac-LTF\$r8AYWAuQL\$S-NH2	1079	1545.86	773.93	773.92
354	Ac-LTF\$r8AYWNleQL\$S-NH2	1080	1573.89	787.95	788.17
355	Ac-LTF\$r8AbuYWAQL\$S-NH2	1081	1545.86	773.93	773.99
356	Ac-LTF\$r8AYWHQL\$S-NH2	1082	1597.87	799.94	799.97
357	Ac-LTF\$r8AYWKQL\$S-NH2	1083	1588.90	795.45	795.53
358	Ac-LTF\$r8AYWQQL\$S-NH2	1084	1574.89	788.45	788.5
359	Ac-LTF\$r8AYWRQL\$S-NH2	1085	1616.91	809.46	809.51

TABLE 2b-continued

Number	Sequence	SEQ ID NO:	Exact Mass	M + 2	Observed mass (m/e)
360	Ac-LTF\$8AYWSQL\$S-NH2	1086	1547.84	774.92	774.96
361	Ac-LTF\$8AYWRAL\$S-NH2	1087	1559.89	780.95	780.95
362	Ac-LTF\$8AYWRQL\$A-NH2	1088	1600.91	801.46	801.52
363	Ac-LTF\$8AYWRAL\$A-NH2	1089	1543.89	772.95	773.03
364	Ac-LTF\$8HYWAQL\$S8S-NH2	1090	1597.87	799.94	799.97
365	Ac-LTF\$HYWAQL\$8S-NH2	1091	1597.87	799.94	799.97
366	Ac-LTF\$8HYWAAL\$S-NH2	1092	1540.84	771.42	771.48
367	Ac-LTF\$8HYWAAbuL\$S-NH2	1093	1554.86	778.43	778.51
368	Ac-LTF\$8HYWALL\$S-NH2	1094	1582.89	792.45	792.49
369	Ac-F\$8AYWHAAL\$A-NH2	1095	1310.72	656.36	656.4
370	Ac-F\$8AYWAAL\$A-NH2	1096	1244.70	623.35	1245.61
371	Ac-F\$8AYWSAL\$A-NH2	1097	1260.69	631.35	1261.6
372	Ac-F\$8AYWRAL\$A-NH2	1098	1329.76	665.88	1330.72
373	Ac-F\$8AYWKAL\$A-NH2	1099	1301.75	651.88	1302.67
374	Ac-F\$8AYWOAL\$A-NH2	1100	1287.74	644.87	1289.13
375	Ac-F\$8VYWEAc3cL\$A-NH2	1101	1342.73	672.37	1343.67
376	Ac-F\$8FYWEAc3cL\$A-NH2	1102	1390.73	696.37	1392.14
377	Ac-F\$8WYWEAc3cL\$A-NH2	1103	1429.74	715.87	1431.44
378	Ac-F\$8RYWEAc3cL\$A-NH2	1104	1399.77	700.89	700.95
379	Ac-F\$8KYWEAc3cL\$A-NH2	1105	1371.76	686.88	686.97
380	Ac-F\$8ANleWEAc3cL\$A-NH2	1106	1264.72	633.36	1265.59
381	Ac-F\$8AVWEAc3cL\$A-NH2	1107	1250.71	626.36	1252.2
382	Ac-F\$8AFWEAc3cL\$A-NH2	1108	1298.71	650.36	1299.64
383	Ac-F\$8AWWEAc3cL\$A-NH2	1109	1337.72	669.86	1338.64
384	Ac-F\$8ARWEAc3cL\$A-NH2	1110	1307.74	654.87	655
385	Ac-F\$8AKWEAc3cL\$A-NH2	1111	1279.73	640.87	641.01
386	Ac-F\$8AYWVAc3cL\$A-NH2	1112	1284.73	643.37	643.38
387	Ac-F\$8AYWFAc3cL\$A-NH2	1113	1332.73	667.37	667.43
388	Ac-F\$8AYWWAc3cL\$A-NH2	1114	1371.74	686.87	686.97
389	Ac-F\$8AYWRAc3cL\$A-NH2	1115	1341.76	671.88	671.94
390	Ac-F\$8AYWKAc3cL\$A-NH2	1116	1313.75	657.88	657.88
391	Ac-F\$8AYWEVL\$A-NH2	1117	1330.73	666.37	666.47
392	Ac-F\$8AYWEFL\$A-NH2	1118	1378.73	690.37	690.44
393	Ac-F\$8AYWEWL\$A-NH2	1119	1417.74	709.87	709.91
394	Ac-F\$8AYWERL\$A-NH2	1120	1387.77	694.89	1388.66
395	Ac-F\$8AYWEKL\$A-NH2	1121	1359.76	680.88	1361.21
396	Ac-F\$8AYWEAc3cL\$V-NH2	1122	1342.73	672.37	1343.59
397	Ac-F\$8AYWEAc3cL\$F-NH2	1123	1390.73	696.37	1392.58
398	Ac-F\$8AYWEAc3cL\$W-NH2	1124	1429.74	715.87	1431.29
399	Ac-F\$8AYWEAc3cL\$R-NH2	1125	1399.77	700.89	700.95
400	Ac-F\$8AYWEAc3cL\$K-NH2	1126	1371.76	686.88	686.97
401	Ac-F\$8AYWEAc3cL\$AV-NH2	1127	1413.77	707.89	707.91
402	Ac-F\$8AYWEAc3cL\$AF-NH2	1128	1461.77	731.89	731.96
403	Ac-F\$8AYWEAc3cL\$AW-NH2	1129	1500.78	751.39	751.5
404	Ac-F\$8AYWEAc3cL\$AR-NH2	1130	1470.80	736.40	736.47
405	Ac-F\$8AYWEAc3cL\$AK-NH2	1131	1442.80	722.40	722.41
406	Ac-F\$8AYWEAc3cL\$AH-NH2	1132	1451.76	726.88	726.93
407	Ac-LTF2NO2\$8HYWAQL\$S-NH2	1133	1642.85	822.43	822.54
408	Ac-LTA\$8HYAAQL\$S-NH2	1134	1406.79	704.40	704.5
409	Ac-LTF\$8HYAAQL\$S-NH2	1135	1482.82	742.41	742.47
410	Ac-QSQQTf\$8NLWALL\$AN-NH2	1136	1966.07	984.04	984.38
411	Ac-QAibQQTF\$8NLWALL\$AN-NH2	1137	1964.09	983.05	983.42
412	Ac-QAibQQTF\$8ALWALL\$AN-NH2	1138	1921.08	961.54	961.59
413	Ac-AAATF\$8AAWAAL\$AA-NH2	1139	1608.90	805.45	805.52
414	Ac-F\$8AAWRAL\$Q-NH2	1140	1294.76	648.38	648.48
415	Ac-TF\$8AAWAAL\$Q-NH2	1141	1310.74	656.37	1311.62
416	Ac-TF\$8AAWRAL\$A-NH2	1142	1338.78	670.39	670.46
417	Ac-VF\$8AAWRAL\$Q-NH2	1143	1393.82	697.91	697.99
418	Ac-AF\$8AAWAAL\$A-NH2	1144	1223.71	612.86	1224.67
420	Ac-TF\$8AAWKAL\$Q-NH2	1145	1367.80	684.90	684.97
421	Ac-TF\$8AAWOAL\$Q-NH2	1146	1353.78	677.89	678.01
422	Ac-TF\$8AAWSAL\$Q-NH2	1147	1326.73	664.37	664.47
423	Ac-LTF\$8AAWRAL\$Q-NH2	1148	1508.89	755.45	755.49
424	Ac-F\$8AYWAQL\$A-NH2	1149	1301.72	651.86	651.96
425	Ac-F\$8AWWAAL\$A-NH2	1150	1267.71	634.86	634.87
426	Ac-F\$8AWWAQL\$A-NH2	1151	1324.73	663.37	663.43
427	Ac-F\$8AYWEAL\$S-NH2	1152	1231.66	616.83	1232.93
428	Ac-F\$8AYWAAL\$S-NH2	1153	1173.66	587.83	1175.09
429	Ac-F\$8AYWKAL\$S-NH2	1154	1230.72	616.36	616.44
430	Ac-F\$8AYWOAL\$S-NH2	1155	1216.70	609.35	609.48
431	Ac-F\$8AYWQAL\$S-NH2	1156	1230.68	616.34	616.44
432	Ac-F\$8AYWAQL\$S-NH2	1157	1230.68	616.34	616.37
433	Ac-F\$8HYWDQL\$S-NH2	1158	1427.72	714.86	714.86
434	Ac-F\$8HFWEQL\$S-NH2	1159	1425.74	713.87	713.98
435	Ac-F\$8AYWHQL\$S-NH2	1160	1383.73	692.87	692.96
436	Ac-F\$8AYWKQL\$S-NH2	1161	1374.77	688.39	688.45
437	Ac-F\$8AYWOQL\$S-NH2	1162	1360.75	681.38	681.49

TABLE 2b-continued

Number	Sequence	SEQ ID NO:	Exact Mass	M + 2	Observed mass (m/e)
438	Ac-F\$8HYWSQL\$S-NH2	1163	1399.73	700.87	700.95
439	Ac-F\$8HWWEQL\$S-NH2	1164	1464.76	733.38	733.44
440	Ac-F\$8HWWAQL\$S-NH2	1165	1406.75	704.38	704.43
441	Ac-F\$8AWWHQL\$S-NH2	1166	1406.75	704.38	704.43
442	Ac-F\$8AWWKQL\$S-NH2	1167	1397.79	699.90	699.92
443	Ac-F\$8AWWOQL\$S-NH2	1168	1383.77	692.89	692.96
444	Ac-F\$8HWWSQL\$S-NH2	1169	1422.75	712.38	712.42
445	Ac-LTF\$8NYWANleL\$Q-NH2	1170	1600.90	801.45	801.52
446	Ac-LTF\$8NLWAQL\$Q-NH2	1171	1565.90	783.95	784.06
447	Ac-LTF\$8NYWANleL\$A-NH2	1172	1543.88	772.94	773.03
448	Ac-LTF\$8NLWAQL\$A-NH2	1173	1508.88	755.44	755.49
449	Ac-LTF\$8AYWANleL\$Q-NH2	1174	1557.90	779.95	780.06
450	Ac-LTF\$8ALWAQL\$Q-NH2	1175	1522.89	762.45	762.45
451	Ac-LAF\$8NYWANleL\$Q-NH2	1176	1570.89	786.45	786.5
452	Ac-LAF\$8NLWAQL\$Q-NH2	1177	1535.89	768.95	769.03
453	Ac-LAF\$8AYWANleL\$A-NH2	1178	1470.86	736.43	736.47
454	Ac-LAF\$8ALWAQL\$A-NH2	1179	1435.86	718.93	719.01
455	Ac-LAF\$8AYWAAL\$A-NH2	1180	1428.82	715.41	715.41
456	Ac-F\$8AYWEAc3cL\$AAib-NH2	1181	1399.75	700.88	700.95
457	Ac-F\$8AYWAQL\$AA-NH2	1182	1372.75	687.38	687.78
458	Ac-F\$8AYWAAc3cL\$AA-NH2	1183	1327.73	664.87	664.84
459	Ac-F\$8AYWSAc3cL\$AA-NH2	1184	1343.73	672.87	672.9
460	Ac-F\$8AYWEAc3cL\$AS-NH2	1185	1401.73	701.87	701.84
461	Ac-F\$8AYWEAc3cL\$AT-NH2	1186	1415.75	708.88	708.87
462	Ac-F\$8AYWEAc3cL\$AL-NH2	1187	1427.79	714.90	714.94
463	Ac-F\$8AYWEAc3cL\$AQ-NH2	1188	1442.76	722.38	722.41
464	Ac-F\$8AFWEAc3cL\$AA-NH2	1189	1369.74	685.87	685.93
465	Ac-F\$8AWWEAc3cL\$AA-NH2	1190	1408.75	705.38	705.39
466	Ac-F\$8AYWEAc3cL\$SA-NH2	1191	1401.73	701.87	701.99
467	Ac-F\$8AYWEAL\$AA-NH2	1192	1373.74	687.87	687.93
468	Ac-F\$8AYWENleL\$AA-NH2	1193	1415.79	708.90	708.94
469	Ac-F\$8AYWEAc3cL\$AbuA-NH2	1194	1399.75	700.88	700.95
470	Ac-F\$8AYWEAc3cL\$NleA-NH2	1195	1427.79	714.90	714.86
471	Ac-F\$8AYWEAibL\$NleA-NH2	1196	1429.80	715.90	715.97
472	Ac-F\$8AYWEAL\$NleA-NH2	1197	1415.79	708.90	708.94
473	Ac-F\$8AYWENleL\$NleA-NH2	1198	1457.83	729.92	729.96
474	Ac-F\$8AYWEAibL\$Abu-NH2	1199	1330.73	666.37	666.39
475	Ac-F\$8AYWENleL\$Abu-NH2	1200	1358.76	680.38	680.39
476	Ac-F\$8AYWEAL\$Abu-NH2	1201	1316.72	659.36	659.36
477	Ac-LTF\$8AFWAQL\$S-NH2	1202	1515.85	758.93	759.12
478	Ac-LTF\$8AWWAQL\$S-NH2	1203	1554.86	778.43	778.51
479	Ac-LTF\$8AYWAQISS-NH2	1204	1531.84	766.92	766.96
480	Ac-LTF\$8AYWAQNleS-NH2	1205	1531.84	766.92	766.96
481	Ac-LTF\$8AYWAQL\$SA-NH2	1206	1602.88	802.44	802.48
482	Ac-LTF\$8AWWAQL\$A-NH2	1207	1538.87	770.44	770.89
483	Ac-LTF\$8AYWAQL\$A-NH2	1208	1515.85	758.93	759.42
484	Ac-LTF\$8AYWAQNleSA-NH2	1209	1515.85	758.93	759.42
485	Ac-LTF\$8AYWAQL\$AA-NH2	1210	1586.89	794.45	794.94
486	Ac-LTF\$8HWWAQL\$S-NH2	1211	1620.88	811.44	811.47
487	Ac-LTF\$8HRWAQL\$S-NH2	1212	1590.90	796.45	796.52
488	Ac-LTF\$8HKWAQL\$S-NH2	1213	1562.90	782.45	782.53
489	Ac-LTF\$8HYWAQL\$W-NH2	1214	1696.91	849.46	849.5
491	Ac-F\$8AYWAbuAL\$A-NH2	1215	1258.71	630.36	630.5
492	Ac-F\$8AbuYWEAL\$A-NH2	1216	1316.72	659.36	659.51
493	Ac-NlePRF%r8NYWRLL%QN-NH2	1217	1954.13	978.07	978.54
494	Ac-TSF%r8HYWAQL%S-NH2	1218	1573.83	787.92	787.98
495	Ac-LTF%r8AYWAQL%S-NH2	1219	1533.86	767.93	768
496	Ac-HTF\$8HYWAQL\$S-NH2	1220	1621.84	811.92	811.96
497	Ac-LHF\$8HYWAQL\$S-NH2	1221	1633.88	817.94	818.02
498	Ac-LTF\$8HHWAQL\$S-NH2	1222	1571.86	786.93	786.94
499	Ac-LTF\$8HYWHQL\$S-NH2	1223	1663.89	832.95	832.38
500	Ac-LTF\$8HYWAHL\$S-NH2	1224	1606.87	804.44	804.48
501	Ac-LTF\$8HYWAQL\$H-NH2	1225	1647.89	824.95	824.98
502	Ac-LTF\$8HYWAQL\$S-NHPr	1226	1639.91	820.96	820.98
503	Ac-LTF\$8HYWAQL\$S-NHsBu	1227	1653.93	827.97	828.02
504	Ac-LTF\$8HYWAQL\$S-NHiBu	1228	1653.93	827.97	828.02
505	Ac-LTF\$8HYWAQL\$S-NHBn	1229	1687.91	844.96	844.44
506	Ac-LTF\$8HYWAQL\$S-NHPe	1230	1700.92	851.46	851.99
507	Ac-LTF\$8HYWAQL\$S-NHChx	1231	1679.94	840.97	841.04
508	Ac-ETF\$8AYWAQL\$S-NH2	1232	1547.80	774.90	774.96
509	Ac-STF\$8AYWAQL\$S-NH2	1233	1505.79	753.90	753.94
510	Ac-LEF\$8AYWAQL\$S-NH2	1234	1559.84	780.92	781.25
511	Ac-LSF\$8AYWAQL\$S-NH2	1235	1517.83	759.92	759.93
512	Ac-LTF\$8EYWAQL\$S-NH2	1236	1589.85	795.93	795.97
513	Ac-LTF\$8SYWAQL\$S-NH2	1237	1547.84	774.92	774.96
514	Ac-LTF\$8AYWEQL\$S-NH2	1238	1589.85	795.93	795.9
515	Ac-LTF\$8AYWEL\$S-NH2	1239	1532.83	767.42	766.96

TABLE 2b-continued

Number	Sequence	SEQ ID NO:	Exact Mass	M + 2	Observed mass (m/e)
516	Ac-LTF\$8AYWASL\$S-NH2	1240	1490.82	746.41	746.46
517	Ac-LTF\$8AYWAQL\$E-NH2	1241	1573.85	787.93	787.98
518	Ac-LTF2CN\$8HYWAQL\$S-NH2	1242	1622.86	812.43	812.47
519	Ac-LTF3C1\$8HYWAQL\$S-NH2	1243	1631.83	816.92	816.99
520	Ac-LTDip\$8HYWAQL\$S-NH2	1244	1673.90	837.95	838.01
521	Ac-LTF\$8HYWAQTL\$S-NH2	1245	1597.87	799.94	800.04
522	Ac-F\$8AY6clWEAL\$A-NH2	1246	1336.66	669.33	1338.56
523	Ac-F\$8AYdl6fWEAL\$A-NH2	1247	1380.61	691.31	692.2
524	Ac-F\$8AYdl6fWEAL\$A-NH2	1248	1320.69	661.35	1321.61
525	Ac-F\$8AYdl4mWEAL\$A-NH2	1249	1316.72	659.36	659.36
526	Ac-F\$8AYdl5clWEAL\$A-NH2	1250	1336.66	669.33	669.35
527	Ac-F\$8AYdl7mWEAL\$A-NH2	1251	1316.72	659.36	659.36
528	Ac-LTF\$8HYWAQL\$%A-NH2	1252	1583.89	792.95	793.01
529	Ac-LTF\$8HCouWAQL\$S-NH2	1253	1679.87	840.94	841.38
530	Ac-LTFEHCouWAQLTS-NH2	1254	1617.75	809.88	809.96
531	Ac-LTA\$8HCouWAQL\$S-NH2	1255	1603.84	802.92	803.36
532	Ac-F\$8AYWEAL\$AbuA-NH2	1256	1387.75	694.88	694.88
533	Ac-F\$8AYWEAISAA-NH2	1257	1373.74	687.87	687.93
534	Ac-F\$8AYWEANle\$A-NH2	1258	1373.74	687.87	687.93
535	Ac-F\$8AYWEAmlL\$A-NH2	1259	1429.80	715.90	715.97
536	Ac-F\$8AYWQAL\$A-NH2	1260	1372.75	687.38	687.48
537	Ac-F\$8AYWAAL\$A-NH2	1261	1315.73	658.87	658.92
538	Ac-F\$8AYWAbuAL\$A-NH2	1262	1329.75	665.88	665.95
539	Ac-F\$8AYWNleAL\$A-NH2	1263	1357.78	679.89	679.94
540	Ac-F\$8AbuYWEAL\$A-NH2	1264	1387.75	694.88	694.96
541	Ac-F\$8NleYWEAL\$A-NH2	1265	1415.79	708.90	708.94
542	Ac-F\$8FYWEAL\$A-NH2	1266	1449.77	725.89	725.97
543	Ac-LTF\$8HYWAQhL\$S-NH2	1267	1611.88	806.94	807
544	Ac-LTF\$8HYWAQAdm\$S-NH2	1268	1675.91	838.96	839.04
545	Ac-LTF\$8HYWAQIgl\$S-NH2	1269	1659.88	830.94	829.94
546	Ac-F\$8AYWAQL\$A-NH2	1270	1372.75	687.38	687.48
547	Ac-LTF\$8ALWAQL\$Q-NH2	1271	1522.89	762.45	762.52
548	Ac-F\$8AYWEAL\$A-NH2	1272	1373.74	687.87	687.93
549	Ac-F\$8AYWENleL\$A-NH2	1273	1415.79	708.90	708.94
550	Ac-F\$8AYWEAibL\$Abu-NH2	1274	1330.73	666.37	666.39
551	Ac-F\$8AYWENleL\$Abu-NH2	1275	1358.76	680.38	680.38
552	Ac-F\$8AYWEAL\$Abu-NH2	1276	1316.72	659.36	659.36
553	Ac-F\$8AYWEAc3cL\$AbuA-NH2	1277	1399.75	700.88	700.95
554	Ac-F\$8AYWEAc3cL\$NleA-NH2	1278	1427.79	714.90	715.01
555	H-LTF\$8AYWAQL\$S-NH2	1279	1489.83	745.92	745.95
556	mdPEG3-LTF\$8AYWAQL\$S-NH2	1280	1679.92	840.96	840.97
557	mdPEG7-LTF\$8AYWAQL\$S-NH2	1281	1856.02	929.01	929.03
558	Ac-F\$8AmpEt6clWEAL\$A-NH2	1282	1470.71	736.36	788.17
559	Ac-LTF3C1\$8AYWAQL\$S-NH2	1283	1565.81	783.91	809.18
560	Ac-LTF3C1\$8HYWAQL\$A-NH2	1284	1615.83	808.92	875.24
561	Ac-LTF3C1\$8HYWWQL\$S-NH2	1285	1746.87	874.44	841.65
562	Ac-LTF3C1\$8AYWWQL\$S-NH2	1286	1680.85	841.43	824.63
563	Ac-LTF\$8AYWWQL\$S-NH2	1287	1646.89	824.45	849.98
564	Ac-LTF\$8HYWWQL\$A-NH2	1288	1696.91	849.46	816.67
565	Ac-LTF\$8AYWWQL\$A-NH2	1289	1630.89	816.45	776.15
566	Ac-LTF4F\$8AYWAQL\$S-NH2	1290	1549.83	775.92	776.15
567	Ac-LTF2F\$8AYWAQL\$S-NH2	1291	1549.83	775.92	776.15
568	Ac-LTF3F\$8AYWAQL\$S-NH2	1292	1549.83	775.92	785.12
569	Ac-LTF34F2\$8AYWAQL\$S-NH2	1293	1567.83	784.92	785.12
570	Ac-LTF35F2\$8AYWAQL\$S-NH2	1294	1567.83	784.92	1338.74
571	Ac-F3C1\$8AYWEAL\$A-NH2	1295	1336.66	669.33	705.28
572	Ac-F3C1\$8AYWEAL\$AA-NH2	1296	1407.70	704.85	680.11
573	Ac-F\$8AY6clWEAL\$AA-NH2	1297	1407.70	704.85	736.83
574	Ac-F\$8AY6clWEAL\$-NH2	1298	1265.63	633.82	784.1
575	Ac-LTF\$8HYWAQL\$S-NH2	1299	16.03	9.02	826.98
576	Ac-LTF\$8HYWAQL\$S-NHsBu	1300	1653.93	827.97	828.02
577	Ac-STF\$8AYWAQL\$S-NH2	1301	1505.79	753.90	753.94
578	Ac-LTF\$8AYWAEL\$S-NH2	1302	1532.83	767.42	767.41
579	Ac-LTF\$8AYWAQL\$E-NH2	1303	1573.85	787.93	787.98
580	mdPEG3-LTF\$8AYWAQL\$S-NH2	1304	1679.92	840.96	840.97
581	Ac-LTF\$8AYWAQhL\$S-NH2	1305	1545.86	773.93	774.31
583	Ac-LTF\$8AYWAQCba\$S-NH2	1306	1571.88	786.94	787.3
584	Ac-LTF\$8AYWAQCbg\$S-NH2	1307	1557.86	779.93	780.4
585	Ac-LTF\$8AYWAQCba\$S-NH2	1308	1543.84	772.92	780.13
586	Ac-LTF\$8AYWAQF\$S-NH2	1309	1565.83	783.92	784.2
587	Ac-LTF4F\$8HYWAQhL\$S-NH2	1310	1629.87	815.94	815.36
588	Ac-LTF4F\$8HYWAQCba\$S-NH2	1311	1655.89	828.95	828.39
589	Ac-LTF4F\$8HYWAQCbg\$S-NH2	1312	1641.87	821.94	821.35
590	Ac-LTF4F\$8HYWAQCba\$S-NH2	1313	1627.86	814.93	814.32
591	Ac-LTF4F\$8AYWAQhL\$S-NH2	1314	1563.85	782.93	782.36
592	Ac-LTF4F\$8AYWAQCba\$S-NH2	1315	1589.87	795.94	795.38
593	Ac-LTF4F\$8AYWAQCbg\$S-NH2	1316	1575.85	788.93	788.35

TABLE 2b-continued

Number	Sequence	SEQ ID NO:	Exact Mass	M + 2	Observed mass (m/e)
594	Ac-LTF4F8r8AYWAQCbaSS-NH2	1317	1561.83	781.92	781.39
595	Ac-LTF3C18r8AYWAQhLSS-NH2	1318	1579.82	790.91	790.35
596	Ac-LTF3C18r8AYWAQChaSS-NH2	1319	1605.84	803.92	803.67
597	Ac-LTF3C18r8AYWAQChgSS-NH2	1320	1591.82	796.91	796.34
598	Ac-LTF3C18r8AYWAQCbaSS-NH2	1321	1577.81	789.91	789.39
599	Ac-LTF8r8AYWAQhFSS-NH2	1322	1579.84	790.92	791.14
600	Ac-LTF8r8AYWAQF3CF3SS-NH2	1323	1633.82	817.91	818.15
601	Ac-LTF8r8AYWAQF3MeSS-NH2	1324	1581.86	791.93	791.32
602	Ac-LTF8r8AYWAQ1Na1SS-NH2	1325	1615.84	808.92	809.18
603	Ac-LTF8r8AYWAQBiPSS-NH2	1326	1641.86	821.93	822.13
604	Ac-LTF8r8FYWAQLSA-NH2	1327	1591.88	796.94	797.33
605	Ac-LTF8r8HYWAQLSS-NHAm	1328	1667.94	834.97	835.92
606	Ac-LTF8r8HYWAQLSS-NHiAm	1329	1667.94	834.97	835.55
607	Ac-LTF8r8HYWAQLSS-NHnPr3Ph	1330	1715.94	858.97	859.79
608	Ac-LTF8r8HYWAQLSS-NHnBu3,3Me	1331	1681.96	841.98	842.49
610	Ac-LTF8r8HYWAQLSS-NHnPr	1332	1639.91	820.96	821.58
611	Ac-LTF8r8HYWAQLSS-NHnEt2Ch	1333	1707.98	854.99	855.35
612	Ac-LTF8r8HYWAQLSS-NHHex	1334	1681.96	841.98	842.4
613	Ac-LTF8r8AYWAQLSS-NHmdPeg2	1335	1633.91	817.96	818.35
614	Ac-LTF8r8AYWAQLSA-NHmdPeg2	1336	1617.92	809.96	810.3
615	Ac-LTF8r8AYWAQLSA-NHmdPeg4	1337	1705.97	853.99	854.33
616	Ac-F8r8AYdl4mWEALSA-NH2	1338	1316.72	659.36	659.44
617	Ac-F8r8AYdl5clWEALSA-NH2	1339	1336.66	669.33	669.43
618	Ac-LThF8r8AYWAQLSS-NH2	1340	1545.86	773.93	774.11
619	Ac-LT2Na18r8AYWAQLSS-NH2	1341	1581.86	791.93	792.43
620	Ac-LTA8r8AYWAQLSS-NH2	1342	1455.81	728.91	729.15
621	Ac-LTF8r8AYWVQLSS-NH2	1343	1559.88	780.94	781.24
622	Ac-LTF8r8HYWAALSA-NH2	1344	1524.85	763.43	763.86
623	Ac-LTF8r8VYWAQLSA-NH2	1345	1543.88	772.94	773.37
624	Ac-LTF8r8IYWAQLSS-NH2	1346	1573.89	787.95	788.17
625	Ac-FTF8r8VYWSQLSS-NH2	1347	1609.85	805.93	806.22
626	Ac-ITF8r8FYWAQLSS-NH2	1348	1607.88	804.94	805.2
627	Ac-2Na1TF8r8VYWSQLSS-NH2	1349	1659.87	830.94	831.2
628	Ac-ITF8r8LYWSQLSS-NH2	1350	1589.89	795.95	796.13
629	Ac-FTF8r8FYWAQLSS-NH2	1351	1641.86	821.93	822.13
630	Ac-WTF8r8VYWAQLSS-NH2	1352	1632.87	817.44	817.69
631	Ac-WTF8r8WYWAQLSS-NH2	1353	1719.88	860.94	861.36
632	Ac-VTF8r8AYWSQLSS-NH2	1354	1533.82	767.91	768.19
633	Ac-WTF8r8FYWSQLSS-NH2	1355	1696.87	849.44	849.7
634	Ac-FTF8r8IYWAQLSS-NH2	1356	1607.88	804.94	805.2
635	Ac-WTF8r8VYWSQLSS-NH2	1357	1648.87	825.44	824.8
636	Ac-FTF8r8LYWSQLSS-NH2	1358	1623.87	812.94	812.8
637	Ac-YTF8r8FYWSQLSS-NH2	1359	1673.85	837.93	837.8
638	Ac-LTF8r8AY6clWEALSA-NH2	1360	1550.79	776.40	776.14
639	Ac-LTF8r8AY6clWSQLSS-NH2	1361	1581.80	791.90	791.68
640	Ac-F8r8AY6clWSALSA-NH2	1362	1294.65	648.33	647.67
641	Ac-F8r8AY6clWQALSA-NH2	1363	1406.72	704.36	703.84
642	Ac-LHF8r8AYWAQLSS-NH2	1364	1567.86	784.93	785.21
643	Ac-LTF8r8AYWAQLSS-NH2	1365	1531.84	766.92	767.17
644	Ac-LTF8r8AHWAQLSS-NH2	1366	1505.84	753.92	754.13
645	Ac-LTF8r8AYWAHLSS-NH2	1367	1540.84	771.42	771.61
646	Ac-LTF8r8AYWAQLSH-NH2	1368	1581.87	791.94	792.15
647	H-LTF8r8AYWAQLSA-NH2	1369	1473.84	737.92	737.29
648	Ac-HHF8r8AYWAQLSS-NH2	1370	1591.83	796.92	797.35
649	Ac-aAibWTF8r8VYWSQLSS-NH2	1371	1804.96	903.48	903.64
650	Ac-AibWTF8r8HYWAQLSS-NH2	1372	1755.91	878.96	879.4
651	Ac-AibAWTF8r8HYWAQLSS-NH2	1373	1826.95	914.48	914.7
652	Ac-fWTF8r8HYWAQLSS-NH2	1374	1817.93	909.97	910.1
653	Ac-AibWWTF8r8HYWAQLSS-NH2	1375	1941.99	972.00	972.2
654	Ac-WTF8r8LYWSQLSS-NH2	1376	1662.88	832.44	832.8
655	Ac-WTF8r8NleYWSQLSS-NH2	1377	1662.88	832.44	832.6
656	Ac-LTF8r8AYWSQLSA-NH2	1378	1531.84	766.92	767.2
657	Ac-LTF8r8EYWARLSA-NH2	1379	1601.90	801.95	802.1
658	Ac-LTF8r8EYWAHLSA-NH2	1380	1582.86	792.43	792.6
659	Ac-aTF8r8AYWAQLSS-NH2	1381	1489.80	745.90	746.08
660	Ac-AibTF8r8AYWAQLSS-NH2	1382	1503.81	752.91	753.11
661	Ac-AmfTF8r8AYWAQLSS-NH2	1383	1579.84	790.92	791.14
662	Ac-AmwTF8r8AYWAQLSS-NH2	1384	1618.86	810.43	810.66
663	Ac-NmLTF8r8AYWAQLSS-NH2	1385	1545.86	773.93	774.11
664	Ac-LNmTF8r8AYWAQLSS-NH2	1386	1545.86	773.93	774.11
665	Ac-LSarF8r8AYWAQLSS-NH2	1387	1501.83	751.92	752.18
667	Ac-LGF8r8AYWAQLSS-NH2	1388	1487.82	744.91	745.15
668	Ac-LTNmF8r8AYWAQLSS-NH2	1389	1545.86	773.93	774.2
669	Ac-TF8r8AYWAQLSS-NH2	1390	1418.76	710.38	710.64
670	Ac-ETF8r8AYWAQLSA-NH2	1391	1531.81	766.91	767.2
671	Ac-LTF8r8EYWAQLSA-NH2	1392	1573.85	787.93	788.1
672	Ac-LT2Na18r8AYWSQLSS-NH2	1393	1597.85	799.93	800.4

TABLE 2b-continued

Number	Sequence	SEQ ID NO:	Exact Mass	M + 2	Observed mass (m/e)
673	Ac-LTFsr8AYWAALSS-NH2	1394	1474.82	738.41	738.68
674	Ac-LTFsr8AYWAQhChaSS-NH2	1395	1585.89	793.95	794.19
675	Ac-LTFsr8AYWAQChgSS-NH2	1396	1557.86	779.93	780.97
676	Ac-LTFsr8AYWAQCbaSS-NH2	1397	1543.84	772.92	773.19
677	Ac-LTFsr8AYWAQF3CF3SS-NH2	1398	1633.82	817.91	818.15
678	Ac-LTFsr8AYWAQ1Na1SS-NH2	1399	1615.84	808.92	809.18
679	Ac-LTFsr8AYWAQBiPSS-NH2	1400	1641.86	821.93	822.32
680	Ac-LT2Na1sr8AYWAQLSS-NH2	1401	1581.86	791.93	792.15
681	Ac-LTFsr8AYWVQLSS-NH2	1402	1559.88	780.94	781.62
682	Ac-LTFsr8AWWAQLSS-NH2	1403	1554.86	778.43	778.65
683	Ac-FTFsr8VYWSQLSS-NH2	1404	1609.85	805.93	806.12
684	Ac-ITFsr8FYWAQLSS-NH2	1405	1607.88	804.94	805.2
685	Ac-ITFsr8LYWSQLSS-NH2	1406	1589.89	795.95	796.22
686	Ac-FTFsr8FYWAQLSS-NH2	1407	1641.86	821.93	822.41
687	Ac-VTFsr8AYWSQLSS-NH2	1408	1533.82	767.91	768.19
688	Ac-LTFsr8AHWAQLSS-NH2	1409	1505.84	753.92	754.31
689	Ac-LTFsr8AYWAQLSH-NH2	1410	1581.87	791.94	791.94
690	Ac-LTFsr8AYWAHLSS-NH2	1411	1540.84	771.42	771.61
691	Ac-aAibWTFsr8VYWSQLSS-NH2	1412	1804.96	903.48	903.9
692	Ac-AibWTFsr8HYWAQLSS-NH2	1413	1755.91	878.96	879.5
693	Ac-AibAWTFsr8HYWAQLSS-NH2	1414	1826.95	914.48	914.7
694	Ac-fWTFsr8HYWAQLSS-NH2	1415	1817.93	909.97	910.2
695	Ac-AibWWTFsr8HYWAQLSS-NH2	1416	1941.99	972.00	972.7
696	Ac-WTFsr8LYWSQLSS-NH2	1417	1662.88	832.44	832.7
697	Ac-WTFsr8NleYWSQLSS-NH2	1418	1662.88	832.44	832.7
698	Ac-LTFsr8AYWSQLSa-NH2	1419	1531.84	766.92	767.2
699	Ac-LTFsr8EYWARLSA-NH2	1420	1601.90	801.95	802.2
700	Ac-LTFsr8EYWAHLSS-NH2	1421	1582.86	792.43	792.6
701	Ac-aTfSr8AYWAQLSS-NH2	1422	1489.80	745.90	746.1
702	Ac-AibTFsr8AYWAQLSS-NH2	1423	1503.81	752.91	753.2
703	Ac-AmfTFsr8AYWAQLSS-NH2	1424	1579.84	790.92	791.2
704	Ac-AmwTFsr8AYWAQLSS-NH2	1425	1618.86	810.43	810.7
705	Ac-NmLTFsr8AYWAQLSS-NH2	1426	1545.86	773.93	774.1
706	Ac-LNmTFsr8AYWAQLSS-NH2	1427	1545.86	773.93	774.4
707	Ac-LSarFsr8AYWAQLSS-NH2	1428	1501.83	751.92	752.1
708	Ac-TFsr8AYWAQLSS-NH2	1429	1418.76	710.38	710.8
709	Ac-ETFsr8AYWAQLSa-NH2	1430	1531.81	766.91	767.4
710	Ac-LTFsr8EYWAQLSa-NH2	1431	1573.85	787.93	788.2
711	Ac-WTFsr8VYWSQLSS-NH2	1432	1648.87	825.44	825.2
713	Ac-YTFsr8FYWSQLSS-NH2	1433	1673.85	837.93	837.3
714	Ac-Fsr8AY6clWSALSS-NH2	1434	1294.65	648.33	647.74
715	Ac-ETFsr8EYWVQLSS-NH2	1435	1633.84	817.92	817.36
716	Ac-ETFsr8EHWAQLSS-NH2	1436	1563.81	782.91	782.36
717	Ac-ITFsr8EYWAQLSS-NH2	1437	1589.85	795.93	795.38
718	Ac-ITFsr8EHWVQLSS-NH2	1438	1575.88	788.94	788.42
719	Ac-ITFsr8EHWAQLSS-NH2	1439	1563.85	782.93	782.43
720	Ac-LTF4Fsr8AYWAQCbaSS-NH2	1440	1561.83	781.92	781.32
721	Ac-LTF3ClSr8AYWAQhLSS-NH2	1441	1579.82	790.91	790.64
722	Ac-LTF3ClSr8AYWAQChaSS-NH2	1442	1605.84	803.92	803.37
723	Ac-LTF3ClSr8AYWAQChgSS-NH2	1443	1591.82	796.91	796.27
724	Ac-LTF3ClSr8AYWAQCbaSS-NH2	1444	1577.81	789.91	789.83
725	Ac-LTFsr8AY6clWSQLSS-NH2	1445	1581.80	791.90	791.75
726	Ac-LTF4Fsr8HYWAQhLSS-NH2	1446	1629.87	815.94	815.36
727	Ac-LTF4Fsr8HYWAQCbaSS-NH2	1447	1627.86	814.93	814.32
728	Ac-LTF4Fsr8AYWAQhLSS-NH2	1448	1563.85	782.93	782.36
729	Ac-LTF4Fsr8AYWAQChgSS-NH2	1449	1575.85	788.93	788.35
730	Ac-ETFsr8EYWVALSS-NH2	1450	1576.82	789.41	788.79
731	Ac-ETFsr8EHWAALSS-NH2	1451	1506.79	754.40	754.8
732	Ac-ITFsr8EYWAALSS-NH2	1452	1532.83	767.42	767.75
733	Ac-ITFsr8EHWVALSS-NH2	1453	1518.86	760.43	760.81
734	Ac-ITFsr8EHWAALSS-NH2	1454	1506.82	754.41	754.8
735	Pam-LTFsr8EYWAQLSS-NH2	1455	1786.07	894.04	894.48
736	Pam-ETFsr8EYWAQLSS-NH2	1456	1802.03	902.02	902.34
737	Ac-LTFsr8AYWLQLSS-NH2	1457	1573.89	787.95	787.39
738	Ac-LTFsr8EYWLQLSS-NH2	1458	1631.90	816.95	817.33
739	Ac-LTFsr8EHWLQLSS-NH2	1459	1605.89	803.95	804.29
740	Ac-LTFsr8VYWAQLSS-NH2	1460	1559.88	780.94	781.34
741	Ac-LTFsr8AYWSQLSS-NH2	1461	1547.84	774.92	775.33
742	Ac-ETFsr8AYWAQLSS-NH2	1462	1547.80	774.90	775.7
743	Ac-LTFsr8EYWAQLSS-NH2	1463	1589.85	795.93	796.33
744	Ac-LTFsr8HYWAQLSS-NHAm	1464	1667.94	834.97	835.37
745	Ac-LTFsr8HYWAQLSS-NHiAm	1465	1667.94	834.97	835.27
746	Ac-LTFsr8HYWAQLSS-NHnPr3Ph	1466	1715.94	858.97	859.42
747	Ac-LTFsr8HYWAQLSS-NHnBu3,3Me	1467	1681.96	841.98	842.67
748	Ac-LTFsr8HYWAQLSS-NHnBu	1468	1653.93	827.97	828.24
749	Ac-LTFsr8HYWAQLSS-NHnPr	1469	1639.91	820.96	821.31
750	Ac-LTFsr8HYWAQLSS-NHnEt2Ch	1470	1707.98	854.99	855.35

TABLE 2b-continued

Number	Sequence	SEQ ID NO:	Exact Mass	M + 2	Observed mass (m/e)
751	Ac-LTF\$8HYWAQL\$S-NHHex	1471	1681.96	841.98	842.4
752	Ac-LTF\$8AYWAQL\$S-NHmdPeg2	1472	1633.91	817.96	855.35
753	Ac-LTF\$8AYWAQL\$A-NHmdPeg2	1473	1617.92	809.96	810.58
754	Ac-LTF\$8AYWAAL\$8S-NH2	1474	1474.82	738.41	738.79
755	Ac-LTF\$8AYWCouQL\$S-NH2	1475	1705.88	853.94	854.61
756	Ac-LTF\$8CouYWAQL\$S-NH2	1476	1705.88	853.94	854.7
757	Ac-CouTF\$8AYWAQL\$S-NH2	1477	1663.83	832.92	833.33
758	H-LTF\$8AYWAQL\$A-NH2	1478	1473.84	737.92	737.29
759	Ac-HHF\$8AYWAQL\$S-NH2	1479	1591.83	796.92	797.72
760	Ac-LT2Nal\$8AYWSQL\$S-NH2	1480	1597.85	799.93	800.68
761	Ac-LTF\$8HCouWAQL\$S-NH2	1481	1679.87	840.94	841.38
762	Ac-LTF\$8AYWCou2QL\$S-NH2	1482	1789.94	895.97	896.51
763	Ac-LTF\$8Cou2YWAQL\$S-NH2	1483	1789.94	895.97	896.5
764	Ac-Cou2TF\$8AYWAQL\$S-NH2	1484	1747.90	874.95	875.42
765	Ac-LTF\$8ACou2WAQL\$S-NH2	1485	1697.92	849.96	850.82
766	Dmaac-LTF\$8AYWAQL\$S-NH2	1486	1574.89	788.45	788.82
767	Hexac-LTF\$8AYWAQL\$S-NH2	1487	1587.91	794.96	795.11
768	Napac-LTF\$8AYWAQL\$S-NH2	1488	1657.89	829.95	830.36
769	Pam-LTF\$8AYWAQL\$S-NH2	1489	1728.06	865.03	865.45
770	Ac-LT2Nal\$8HYAAQL\$S-NH2	1490	1532.84	767.42	767.61
771	Ac-LT2Nal\$8HYWAQL\$S-NH2	1491	1675.91	838.96	839.1
772	Ac-LT2Nal\$8HYFAQL\$S-NH2	1492	1608.87	805.44	805.9
773	Ac-LT2Nal\$8HWAAQL\$S-NH2	1493	1555.86	778.93	779.08
774	Ac-LT2Nal\$8HYAWQL\$S-NH2	1494	1647.88	824.94	825.04
775	Ac-LT2Nal\$8HYAAQW\$S-NH2	1495	1605.83	803.92	804.05
776	Ac-LTW\$8HYWAQL\$S-NH2	1496	1636.88	819.44	819.95
777	Ac-LT1Nal\$8HYWAQL\$S-NH2	1497	1647.88	824.94	825.41
778	Ac-F\$8ApmPEt6clWEAL\$A-NH2	1502	1470.71	736.36	788.17

In some embodiments, a peptidomimetic macrocycles disclosed herein do not comprise a peptidomimetic macrocycle structure as shown in Table 2b.

Table 2c shows examples of non-crosslinked polypeptides comprising D-amino acids.

ing concentrated (3.4 μ M) malonate followed by flash cooling, storage, and shipment in liquid nitrogen.

Data collection was performed at the APS at beamline 31-ID (SGX-CAT) at 100° K and wavelength 0.97929 Å. The beamline was equipped with a Rayonix 225-HE detec-

SP	Sequence	SEQ ID NO:	Isomer	Exact Mass	Found Mass	Calc (M + 1)/1	Calc (M + 2)/2	Calc (M + 3)/3
SP765	Ac-tawyanfekllr-NH2	1498			777.46			
SP766	Ac-tawyanf4CF3ekllr-NH2	1499			811.41			

Example 3: X-Ray Co-Crystallography of Peptidomimetic Macrocycles in Complex with MDMX

For co-crystallization with peptide 46 (Table 2b), a stoichiometric amount of compound from a 100 mM stock solution in DMSO was added to the zebrafish MDMX protein solution and allowed to sit overnight at 4° C. before setting up crystallization experiments. Procedures were similar to those described by Popowicz et al. with some variations, as noted below. Protein (residues 15-129, L46V/V95L) was obtained from an *E. coli* BL21(DE3) expression system using the pET15b vector. Cells were grown at 37° C. and induced with 1 mM IPTG at an OD₆₀₀ of 0.7. Cells were allowed to grow an additional 18 hr at 23° C. Protein was purified using Ni-NT Agarose followed by Superdex 75 buffered with 50 mM NaPO₄, pH 8.0, 150 mM NaCl, 2 mM TCEP and then concentrated to 24 mg/ml. The buffer was exchanged to 20 mM Tris, pH 8.0, 50 mM NaCl, 2 mM DTT for crystallization experiments. Initial crystals were obtained with the Nextal (Qiagen) AMS screen #94 and the final optimized reservoir was 2.6 μ M AMS, 75 mM Hepes, pH 7.5. Crystals grew routinely as thin plates at 4° C. and were cryo-protected by pulling them through a solution contain-

tor. For data collection, crystals were rotated through 180° in 1° increments using 0.8 second exposure times. Data were processed and reduced using Mosflm/scala (CCP4; see The CCP4 Suite: Programs for Protein Crystallography. Acta Crystallogr. D50, 760-763 (1994); P. R. Evans. Joint CCP4 and ESF-EACBM Newsletter 33, 22-24 (1997)) in space group C2 (unit cell: a=109.2786, b=81.0836, c=30.9058 Å, a=90, Bf=89.8577, y=90°). Molecular replacement with program Molrep (CCP4; see A. Vagin & A. Teplyakov. J. Appl. Cryst. 30, 1022-1025 (1997)) was performed with the MDMX component of the structure determined by Popowicz et al. (2Z5S; see G. M. Popowicz, A. Czarna, U. Rothweiler, A. Szwagierczak, M. Krajewski, L. Weber & T. A. Holak. Cell Cycle 6, 2386-2392 (2007)) and identified two molecules in the asymmetric unit. Initial refinement of just the two molecules of the zebrafish MDMX with program Refmac (CCP4; see G. N. Murshudov, A. A. Vagin & E. J. Dodson. Acta Crystallogr. D53, 240-255 (1997)) resulted in an R-factor of 0.3424 (R_{free}=0.3712) and rmsd values for bonds (0.018 Å) and angles (1.698°). The electron density for the stapled peptide components, starting with Gln¹⁹ and including all of the aliphatic staple, was very clear. Further refinement with CNX (Accelrys) using data to 2.3 Å resolution resulted in a model (comprised of 1448 atoms

137

from MDMX, 272 atoms from the stapled peptides and 46 water molecules) that is well refined ($R_g=0.2601$, $R_{free}=0.3162$, rmsd bonds=0.007 Å and rmsd angles=0.916°).

Results from this Example are shown in FIGS. 1 and 2. 5

Example 4: Circular Dichroism (CD) Analysis of Alpha-Helicity

Peptide solutions were analyzed by CD spectroscopy using a Jasco J-815 spectropolarimeter (Jasco Inc., Easton, Md.) with the Jasco Spectra Manager Ver.2 system software. A Peltier temperature controller was used to maintain temperature control of the optical cell. Results are expressed as mean molar ellipticity $[\theta]$ (deg cm² dmol⁻¹) as calculated from the equation $[\theta]=0 \text{ obs-MRW}/10 \times 1^\circ \text{c}$ where Oobs is the observed ellipticity in millidegrees, MRW is the mean residue weight of the peptide (peptide molecular weight/number of residues), 1 is the optical path length of the cell in centimeters, and c is the peptide concentration in mg/ml. Peptide concentrations were determined by amino acid analysis. Stock solutions of peptides were prepared in benign CD buffer (20 mM phosphoric acid, pH 2). The stocks were used to prepare peptide solutions of 0.05 mg/ml in either benign CD buffer or CD buffer with 50% trifluoroethanol (TFE) for analyses in a 10 mm path length cell. Variable wavelength measurements of peptide solutions were scanned at 4° C. from 195 to 250 nm, in 0.2 nm increments, and a scan rate 50 nm per minute. The average of six scans was reported. 10 15 20 25 30

Table 3 shows circular dichroism data for selected peptidomimetic macrocycles:

TABLE 3

SP#	Molar Ellip- ticity Benign (222 in 0% TFE)	Molar Ellip- ticity 50% TFE (222 in 50% TFE)	Molar Ellip- ticity TFE - Molar Ellipticity Benign	% Helix 50% TFE compared to 50% TFE parent (CD)	% Helix benign compared to 50% TFE parent (CD)
7	124	-19921.4	-20045.4	137.3	-0.9
11	-398.2	-16623.4	16225.2	106.1	2.5
41	-909	-21319.4	20410.4	136	5.8
43	-15334.5	-18247.4	2912.9	116.4	97.8
69	-102.6	-21509.7	-21407.1	148.2	0.7
71	-121.2	-17957	-17835.9	123.7	0.8
154	-916.2	-30965.1	-30048.9	213.4	6.3
230	-213.2	-17974	-17760.8	123.9	1.5
233	-477.9	-19032.6	-18554.7	131.2	3.3

Example 5: Direct Binding Assay MDM2 with Fluorescence Polarization (FP)

The assay was performed according to the following general protocol: 55

1. Dilute MDM2 (In-house, 41 kD) into FP buffer (High salt buffer-200 mM NaCl, 5 mM CHAPS, pH 7.5) to make 10 μM working stock solution.
2. Add 30 μl of 10 μM of protein stock solution into A1 and B1 well of 96-well black HE microplate (Molecular Devices).
3. Fill in 30 μl of FP buffer into column A2 to A12, B2 to B12, C1 to C12, and D1 to D12.
4. 2 or 3 fold series dilution of protein stock from A1, B1 into A2, B2; A2, B2 to A3, B3; . . . to reach the single digit nM concentration at the last dilution point. 65

138

5. Dilute 1 mM (in 100% DMSO) of FAM labeled linear peptide with DMSO to 100 μM (dilution 1:10). Then, dilute from 100 μM to 10 μM with water (dilution 1:10) and then dilute with FP buffer from 10 μM to 40 nM (dilution 1:250). This is the working solution which will be a 10 nM concentration in well (dilution 1:4). Keep the diluted FAM labeled peptide in the dark until use.
6. Add 10 μl of 10 nM of FAM labeled peptide into each well and incubate, and read at different time points. Kd with 5-FAM-BaLTFEHYWAQLTS-NH₂ (SEQ ID NO: 943) is ~13.38 nM.

Example 6: Competitive Fluorescence Polarization Assay for MDM2

The assay was performed according to the following general protocol:

1. Dilute MDM2 (In-house, 41 kD) into FP buffer (High salt buffer-200 mM NaCl, 5 mM CHAPS, pH 7.5) to make 84 nM (2×) working stock solution.
2. Add 201 μl of 84 nM (2×) of protein stock solution into each well of 96-well black HE microplate (Molecular Devices)
3. Dilute 1 mM (in 100% DMSO) of FAM labeled linear peptide with DMSO to 100 μM (dilution 1:10). Then, dilute from 100 μM to 10 μM with water (dilution 1:10) and then dilute with FP buffer from 10 μM to 40 nM (dilution 1:250). This is the working solution which will be a 10 nM concentration in well (dilution 1:4). Keep the diluted FAM labeled peptide in the dark until use.
4. Make unlabeled peptide dose plate with FP buffer starting with 1 μM (final) of peptide and making 5 fold serial dilutions for 6 points using following dilution scheme. Dilute 10 mM (in 100% DMSO) with DMSO to 5 mM (dilution 1:2). Then, dilute from 5 mM to 500 μM with H₂O (dilution 1:10) and then dilute with FP buffer from 500 μM to 20 μM (dilution 1:25). Making 5 fold serial dilutions from 4 μM (4×) for 6 points.
5. Transfer 10 μl of serial diluted unlabeled peptides to each well which is filled with 20 μl of 84 nM of protein.
6. Add 10 μl of 10 nM (4×) of FAM labeled peptide into each well and incubate for 3 hr to read. 35 40 45

Example 7: Direct Binding Assay MDMX with Fluorescence Polarization (FP)

The assay was performed according to the following general protocol:

1. Dilute MDMX (In-house, 40 kD) into FP buffer (High salt buffer-200 mM NaCl, 5 mM CHAPS, pH 7.5) to make 10 μM working stock solution.
2. Add 301 μl of 10 μM of protein stock solution into A1 and B1 well of 96-well black HE microplate (Molecular Devices).
3. Fill in 30 μl of FP buffer into column A2 to A12, B2 to B12, C1 to C12, and D1 to D12.
4. 2 or 3 fold series dilution of protein stock from A1, B1 into A2, B2; A2, B2 to A3, B3; . . . to reach the single digit nM concentration at the last dilution point.
5. Dilute 1 mM (in 100% DMSO) of FAM labeled linear peptide with DMSO to 100 μM (dilution 1:10). Then, dilute from 100 μM to 10 μM with water (dilution 1:10) and then dilute with FP buffer from 10 μM to 40 nM (dilution 1:250). This is the working solution which 50

139

will be a 10 nM concentration in well (dilution 1:4).
Keep the diluted FAM labeled peptide in the dark until use.

6. Add 10 μ l of 10 nM of FAM labeled peptide into each well and incubate, and read at different time points.
Kd with 5-FAM-BaLTFEHYWAQLTS-NH₂ (SEQ ID NO: 943) is ~51 nM.

Example 8: Competitive Fluorescence Polarization
Assay for MDMX

The assay was performed according to the following general protocol:

1. Dilute MDMX (In-house, 40 kD) into FP buffer (High salt buffer-200 mM NaCl, 5 mM CHAPS, pH 7.5) to make 300 nM (2 $\times$) working stock solution.
2. Add 20 μ l of 300 nM (2 $\times$) of protein stock solution into each well of 96-well black HE microplate (Molecular Devices)
3. Dilute 1 mM (in 100% DMSO) of FAM labeled linear peptide with DMSO to 100 μ M (dilution 1:10). Then, dilute from 100 μ M to 10 μ M with water (dilution 1:10) and then dilute with FP buffer from 10 μ M to 40 nM (dilution 1:250). This is the working solution which will be a 10 nM concentration in well (dilution 1:4). Keep the diluted FAM labeled peptide in the dark until use.
4. Make unlabeled peptide dose plate with FP buffer starting with 5 μ M (final) of peptide and making 5 fold serial dilutions for 6 points using following dilution scheme.
5. Dilute 10 mM (in 100% DMSO) with DMSO to 5 mM (dilution 1:2). Then, dilute from 5 mM to 500 μ M with H₂O (dilution 1:10) and then dilute with FP buffer from 500 μ M to 20 μ M (dilution 1:25). Making 5 fold serial dilutions from 20 μ M (4 $\times$) for 6 points.
6. Transfer 10 μ l of serial diluted unlabeled peptides to each well which is filled with 20 μ l of 300 nM of protein.
7. Add 10 μ l of 10 nM (4 $\times$) of FAM labeled peptide into each well and incubate for 3 hr to read. Results from Examples 5-8 are shown in Table 4. The following scale is used: “+” represents a value greater than 1000 nM, “++” represents a value greater than 100 and less than or equal to 1000 nM, “+++” represents a value greater than 10 nM and less than or equal to 100 nM, and “++++” represents a value of less than or equal to 10 nM.

TABLE 4

SP#	IC50 (MDM2)	IC50 (MDMX)	Ki (MDM2)	Ki (MDMX)
3	++	++	+++	+++
4	+++	++	++++	+++
5	+++	++	++++	+++
6	++	++	+++	+++
7	+++	+++	++++	+++
8	++	++	+++	+++
9	++	++	+++	+++
10	++	++	+++	+++
11	+++	++	++++	+++
12	+	+	+++	++
13	++	++	+++	++
14	+++	+++	++++	++++
15	+++	++	+++	+++
16	+++	+++	++++	+++
17	+++	+++	++++	+++
18	+++	+++	++++	++++
19	++	+++	+++	+++
20	++	++	+++	+++

140

TABLE 4-continued

SP#	IC50 (MDM2)	IC50 (MDMX)	Ki (MDM2)	Ki (MDMX)
21	++	+++	+++	+++
22	+++	+++	++++	+++
23	++	++	+++	+++
24	+++	++	++++	+++
26	+++	++	++++	+++
28	+++	+++	++++	+++
30	++	++	+++	+++
32	+++	++	++++	+++
38	+	++	++	+++
39	+	++	++	++
40	++	++	++	+++
41	++	+++	+++	+++
42	++	++	+++	++
43	+++	+++	++++	+++
45	+++	+++	++++	++++
46	+++	+++	++++	+++
47	++	++	+++	+++
48	++	++	+++	+++
49	++	++	+++	+++
50	+++	++	++++	+++
52	+++	+++	++++	++++
54	++	++	+++	+++
55	+	+	++	++
65	+++	++	++++	+++
68	++	++	+++	+++
69	+++	++	++++	+++
70	++	++	++++	+++
71	+++	++	++++	+++
75	+++	++	++++	+++
77	+++	++	++++	+++
80	+++	++	++++	+++
81	++	++	+++	+++
82	++	++	+++	+++
85	+++	++	++++	+++
99	++++	++	++++	+++
100	++	++	++++	+++
101	+++	++	++++	+++
102	++	++	++++	+++
103	++	++	++++	+++
104	+++	++	++++	+++
105	+++	++	++++	+++
106	++	++	+++	+++
107	++	++	+++	+++
108	+++	++	++++	+++
109	+++	++	++++	+++
110	++	++	++++	+++
111	++	++	++++	+++
112	++	++	+++	+++
113	++	++	+++	+++
114	+++	++	++++	+++
115	++++	++	++++	+++
116	+	+	++	++
118	++++	++	++++	+++
120	+++	++	++++	+++
121	++++	++	++++	+++
122	++++	++	++++	+++
123	++++	++	++++	+++
124	++++	++	++++	+++
125	++++	++	++++	+++
126	++++	++	++++	+++
127	++++	++	++++	+++
128	++++	++	++++	+++
129	++++	++	++++	+++
130	++++	++	++++	+++
133	++++	++	++++	+++
134	++++	++	++++	+++
135	++++	++	++++	+++
136	++++	++	++++	+++
137	++++	++	++++	+++
139	++++	++	++++	+++
142	++++	+++	++++	+++
144	++++	++	++++	+++
146	++++	++	++++	+++
148	++++	++	++++	+++
150	++++	++	++++	+++
153	++++	+++	++++	+++
154	++++	+++	++++	++++
156	++++	++	++++	+++

141

TABLE 4-continued

SP#	IC50 (MDM2)	IC50 (MDMX)	Ki (MDM2)	Ki (MDMX)
158	++++	++	++++	+++
160	++++	++	++++	+++
161	++++	++	++++	+++
166	++++	++	++++	+++
167	+++	++	++++	++
169	++++	+++	++++	+++
170	++++	++	++++	+++
173	++++	++	++++	+++
175	++++	++	++++	+++
177	+++	++	++++	+++
180	+++	++	++++	+++
182	++++	++	++++	+++
185	+++	+	++++	++
186	+++	++	++++	+++
189	+++	++	++++	+++
192	+++	++	++++	+++
194	+++	++	++++	++
196	+++	++	++++	+++
197	++++	++	++++	+++
199	+++	++	++++	++
201	+++	++	++++	++
203	+++	++	++++	+++
204	+++	++	++++	+++
206	+++	++	++++	+++
207	++++	++	++++	+++
210	++++	++	++++	+++
211	++++	++	++++	+++
213	++++	++	++++	+++
215	+++	++	++++	+++
217	++++	++	++++	+++
218	++++	++	++++	+++
221	++++	+++	++++	+++
227	++++	++	++++	+++
230	++++	+++	++++	++++
232	++++	++	++++	+++
233	++++	+++	++++	+++
236	+++	++	++++	+++
237	+++	++	++++	+++
238	+++	+++	++++	+++
239	+++	++	+++	+++
240	+++	++	++++	+++
241	+++	++	++++	+++
242	+++	++	++++	+++
243	+++	+++	++++	+++
244	+++	+++	++++	++++
245	+++	+++	++++	+++
246	+++	++	++++	+++
247	+++	+++	++++	+++
248	+++	+++	++++	+++
249	+++	+++	++++	++++
250	++	+	++	+
252	++	+	++	+
254	+++	++	++++	+++
255	+++	+++	++++	+++
256	+++	+++	++++	+++
257	+++	+++	++++	+++
258	+++	++	++++	+++
259	+++	+++	++++	+++
260	+++	+++	++++	+++
261	+++	++	++++	+++
262	+++	++	++++	+++
263	+++	++	++++	+++
264	+++	+++	++++	+++
266	+++	++	++++	+++
267	+++	+++	++++	++++
270	++++	+++	++++	+++
271	++++	+++	++++	++++
272	++++	+++	++++	++++
276	+++	+++	++++	++++
277	+++	+++	++++	++++
278	+++	+++	++++	++++
279	++++	+++	++++	+++
280	+++	++	++++	+++
281	+++	+	+++	++
282	++	+	+++	+
283	+++	++	+++	++
284	+++	++	++++	+++
289	+++	+++	++++	+++

142

TABLE 4-continued

SP#	IC50 (MDM2)	IC50 (MDMX)	Ki (MDM2)	Ki (MDMX)
291	+++	+++	++++	++++
293	++++	+++	++++	+++
306	++++	++	++++	+++
308	++	++	+++	+++
310	+++	+++	++++	+++
312	+++	++	+++	+++
313	++++	++	++++	+++
314	++++	+++	++++	++++
315	+++	+++	++++	+++
316	++++	++	++++	+++
317	+++	++	+++	+++
318	+++	++	+++	+++
319	+++	++	+++	++
320	+++	++	+++	++
321	+++	++	++++	+++
322	+++	++	+++	++
323	+++	+	+++	++
328	+++	+++	++++	+++
329	+++	+++	++++	+++
331	++++	+++	++++	++++
332	++++	+++	++++	++++
334	++++	+++	++++	++++
336	++++	+++	++++	++++
339	++++	++	++++	+++
341	+++	+++	++++	++++
343	+++	+++	++++	++++
347	+++	+++	++++	+++
349	++++	+++	++++	++++
351	++++	+++	++++	++++
353	++++	+++	++++	++++
355	++++	+++	++++	++++
357	++++	+++	++++	++++
359	++++	+++	++++	+++
360	++++	++++	++++	++++
363	+++	+++	++++	++++
364	+++	+++	++++	++++
365	+++	+++	++++	++++
366	+++	+++	++++	++++
369	++	++	+++	+++
370	+++	+++	++++	+++
371	++	++	+++	+++
372	++	++	+++	+++
373	+++	+++	+++	+++
374	+++	+++	++++	++++
375	+++	+++	++++	++++
376	+++	+++	++++	++++
377	+++	+++	++++	+++
378	+++	+++	++++	+++
379	+++	+++	++++	+++
380	+++	+++	++++	+++
381	+++	+++	++++	+++
382	+++	+++	++++	++++
384	++	+	++	+
386	++	+	++	+
388	++	+++	+++	++++
390	+++	+++	++++	+++
392	+++	+++	++++	++++
394	++++	+++	++++	++++
396	++++	++++	++++	++++
398	+++	+++	++++	+++
402	++++	++++	++++	++++
404	+++	+++	++++	++++
408	+++	+++	++++	+++
410	++++	++++	++++	++++
411	++	+	++	+
412	++++	+++	++++	++++
415	++++	++++	++++	++++
416	+++	+++	++++	+++
417	+++	+++	++++	+++
418	++++	+++	++++	++++
419	+++	+++	+++	++++
421	++++	++++	++++	++++
423	+++	+++	++++	+++
425	+++	+++	+++	+++
427	++	++	+++	+++
432	++++	+++	++++	++++
434	+++	+++	++++	+++
435	++++	+++	++++	++++

TABLE 4-continued

SP#	IC50 (MDM2)	IC50 (MDMX)	Ki (MDM2)	Ki (MDMX)
437	+++	+++	++++	+++
439	++++	+++	++++	++++
441	++++	++++	++++	++++
443	+++	+++	++++	+++
445	+++	++	++++	+++
446	+++	+	++++	+
447	++	+	++	+
551	N/A	N/A	++++	+++
555	N/A	N/A	++++	+++
556	N/A	N/A	++++	+++
557	N/A	N/A	+++	+++
558	N/A	N/A	+++	+++
559	N/A	N/A	+++	+++
560	N/A	N/A	+	+
561	N/A	N/A	++++	+++
562	N/A	N/A	+++	+++
563	N/A	N/A	+++	+++
564	N/A	N/A	++++	+++
565	N/A	N/A	+++	+++
566	N/A	N/A	++++	+++
567	N/A	N/A	++++	+++
568	N/A	N/A	++++	++++
569	N/A	N/A	++++	+++
570	N/A	N/A	++++	+++
571	N/A	N/A	++++	+++
572	N/A	N/A	+++	+++
573	N/A	N/A	+++	+++
574	N/A	N/A	++++	+++
575	N/A	N/A	++++	+++
576	N/A	N/A	++++	+++
577	N/A	N/A	++++	+++
578	N/A	N/A	++++	+++
585	N/A	N/A	+++	+++
586	N/A	N/A	++++	+++
587	N/A	N/A	++++	++++
589	N/A	N/A	++++	++++
594	N/A	N/A	++++	++++
596	N/A	N/A	++++	+++
597	N/A	N/A	++++	+++
598	N/A	N/A	++++	+++
600	N/A	N/A	++++	++++
602	N/A	N/A	++++	++++
603	N/A	N/A	++++	++++
604	N/A	N/A	+++	+++
608	N/A	N/A	++++	+++
609	N/A	N/A	++++	+++
610	N/A	N/A	++++	+++
611	N/A	N/A	++++	+++
612	N/A	N/A	++++	+++
613	N/A	N/A	++++	+++
615	N/A	N/A	++++	++++
433	N/A	N/A	++++	+++
686	N/A	N/A	++++	+++
687	N/A	N/A	++	++
595	N/A	N/A	+	N/A
665	N/A	N/A	+++	N/A
708	N/A	N/A	+++	+++
710	N/A	N/A	+++	+++
711	N/A	N/A	+++	++
712	N/A	N/A	++++	++++
713	N/A	N/A	++++	++++
716	N/A	N/A	++++	++++
765	+	+		
766	+++	+		
752	++	+		
753	+++	+		
754	++	+		
755	++++	+		
756	+++	+		
757	++++	+		
758	+++	+		

Example 9: Competition Binding ELISA (MDM2 & MDMX)

p53-His6 protein ("His6" disclosed as SEQ ID NO: 1501) (30 nM/well) is coated overnight at room temperature in the

wells of a 96-well Immulon plates. On the day of the experiment, plates are washed with 1×PBS-Tween 20 (0.05%) using an automated ELISA plate washer, blocked with ELISA Micro well Blocking for 30 minutes at room temperature; excess blocking agent is washed off by washing plates with 1×PBS-Tween 20 (0.05%). Peptides are diluted from 10 mM DMSO stocks to 500 μ M working stocks in sterile water, further dilutions made in 0.5% DMSO to keep the concentration of DMSO constant across the samples. The peptides are added to wells at 2× desired concentrations in 50 μ L volumes, followed by addition of diluted GST-MDM2 or GST-HMDX protein (final concentration: 10 nM). Samples are incubated at room temperature for 2 h, plates are washed with PBS-Tween 20 (0.05%) prior to adding 100 μ L of HRP-conjugated anti-GST antibody [Hypromatrix, INC] diluted to 0.5 μ g/ml in HRP-stabilizing buffer. Post 30 min incubation with detection antibody, plates are washed and incubated with 100 μ L per well of TMB-E Substrate solution up to 30 minutes; reactions are stopped using 1 μ M HCL and absorbance measured at 450 nm on micro plate reader. Data is analyzed using Graph Pad PRISM software.

Example 10: Cell Viability Assay

The assay was performed according to the following general protocol:

Cell Plating: Trypsinize, count and seed cells at the pre-determined densities in 96-well plates a day prior to assay. Following cell densities are used for each cell line in use:

SJSA-1: 7500 cells/well
RKO: 5000 cells/well
RKO-E6: 5000 cells/well
HCT-116: 5000 cells/well
SW-480: 2000 cells/well
MCF-7: 5000 cells/well

On the day of study, replace media with fresh media with 11% FBS (assay media) at room temperature. Add 180 μ L of the assay media per well. Control wells with no cells, receive 200 μ L media.

Peptide dilution: all dilutions are made at room temperature and added to cells at room temperature.

Prepare 10 mM stocks of the peptides in DMSO. Serially dilute the stock using 1:3 dilution scheme to get 10, 3.3, 1.1, 0.33, 0.11, 0.03, 0.01 mM solutions using DMSO as diluents. Dilute the serially DMSO-diluted peptides 33.3 times using sterile water. This gives range of 10× working stocks. Also prepare DMSO/sterile water (3% DMSO) mix for control wells.

Thus the working stocks concentration range μ M will be 300, 100, 30, 10, 3, 1, 0.3 and 0 μ M. Mix well at each dilution step using multichannel.

Row H has controls. H1-H3 will receive 20 μ L of assay media. H4-H9 will receive 20 μ L of 3% DMSO-water vehicle. H10-H12 will have media alone control with no cells.

Positive control: MDM2 small molecule inhibitor, Nutlin-3a (10 mM) is used as positive control. Nutlin was diluted using the same dilution scheme as peptides.

Addition of working stocks to cells:

Add 20 μ L of 10× desired concentration to appropriate well to achieve the final concentrations in total 200 μ L volume in well. (20 μ L of 300 μ M peptide+180 μ L of cells in media=30 μ M final concentration in 200 μ L volume in wells). Mix gently a few times using pipette.

145

Thus final concentration range used will be 30, 10, 3, 1, 0.3, 0.1, 0.03 & 0 μ M (for potent peptides further dilutions are included).

Controls include wells that get no peptides but contain the same concentration of DMSO as the wells containing the peptides, and wells containing NO CELLS.

Incubate for 72 hours at 37° C. in humidified 5% CO₂ atmosphere.

The viability of cells is determined using MTT reagent from Promega. Viability of SJSA-1, RKO, RKO-E6, HCT-116 cells is determined on day 3, MCF-7 cells on day 5 and SW-480 cells on day 6. At the end of designated incubation time, allow the plates to come to room temperature. Remove 80 μ l of assay media from each well. Add 15 μ l of thawed MTT reagent to each well.

Allow plate to incubate for 2 h at 37° C. in humidified 5% CO₂ atmosphere and add 100 μ l solubilization reagent as per manufacturer's protocol. Incubate with agitation for 1 h at room temperature and read on Synergy Biotek multiplate reader for absorbance at 570 nM.

Analyze the cell viability against the DMSO controls using GraphPad PRISM analysis tools.

Reagents:

Invitrogen cell culture Media

i. Falcon 96-well clear cell culture treated plates (Nunc 353072)

DMSO (Sigma D 2650)

RPMI 1640 (Invitrogen 72400)

MTT (Promega G4000)

Instruments: Multiplate Reader for Absorbance readout (Synergy 2).

Results from cell viability assays are shown in Tables 5 and 6. The following scale is used: "+" represents a value greater than 30 μ M, "++" represents a value greater than 15 μ M and less than or equal to 30 μ M, "+++" represents a value greater than 5 μ M and less than or equal to 15 μ M, and "++++" represents a value of less than or equal to 5 μ M. "IC50 ratio" represents the ratio of average IC50 in p53+/+ cells relative to average IC50 in p53-/- cells.

TABLE 5

SP#	SJSA-1 EC50 (72 h)	
3	+++	45
4	+++	
5	++++	
6	++	
7	++++	
8	+++	
9	+++	50
10	+++	
11	++++	
12	++	
13	+++	
14	+	
15	++	55
16	+	
17	+	
18	+	
19	++	
20	+	
21	+	60
22	+	
24	+++	
26	++++	
28	+	
29	+	
30	+	
32	++	65
38	+	

146

TABLE 5-continued

SP#	SJSA-1 EC50 (72 h)
39	+
40	+
41	+
42	+
43	++
45	+
46	+
47	+
48	+
49	+++
50	++++
52	+
54	+
55	+
65	++++
68	++++
69	++++
70	++++
71	++++
72	++++
74	++++
75	++++
77	++++
78	++
80	++++
81	+++
82	+++
83	+++
84	+
85	+++
99	++++
102	+++
103	+++
104	+++
105	+++
108	+++
109	+++
110	+++
111	++
114	++++
115	++++
118	++++
120	++++
121	++++
122	++++
123	++++
124	+++
125	++++
126	++++
127	++++
128	+++
129	++
130	++++
131	+++
132	++++
133	+++
134	+++
135	+++
136	++
137	+++
139	++++
142	+++
144	++++
147	++++
148	++++
149	++++
150	++++
152	+++
153	++++
154	++++
155	++
156	+++
157	+++
158	+++
160	++++
161	++++
162	+++
163	+++

147

TABLE 5-continued

SP#	SJSA-1 EC50 (72 h)	
166	++	
167	+++	5
168	++	
169	++++	
170	++++	
171	++	
173	+++	
174	++++	10
175	+++	
176	+++	
177	++++	
179	+++	
180	+++	
181	+++	15
182	++++	
183	++++	
184	+++	
185	+++	
186	++	
188	++	
190	++++	20
192	+++	
193	++	
194	+	
195	++++	
196	++++	
197	++++	25
198	++	
199	+++	
200	+++	
201	++++	
202	+++	
203	++++	30
204	++++	
205	++	
206	++	
207	+++	
208	+++	
209	++++	35
210	+++	
211	++++	
213	++++	
214	++++	
215	++++	
216	++++	40
217	++++	
218	++++	
219	++++	
220	+++	
221	++++	
222	+++	
223	++++	45
224	++	
225	+++	
226	++	
227	+++	
228	++++	
229	++++	50
230	++++	
231	++++	
232	++++	
233	++++	
234	++++	
235	++++	55
236	++++	
237	++++	
238	++++	
239	+++	
240	++	
241	+++	
242	++++	60
243	++++	
244	++++	
245	++++	
246	+++	
247	++++	
248	++++	65
249	++++	

148

TABLE 5-continued

SP#	SJSA-1 EC50 (72 h)	
250	++	
251	+	
252	+	
253	+	
254	+++	
255	+++	
256	++	
257	+++	
258	+++	
259	++	
260	++	
261	++	
262	+++	
263	++	
264	++++	
266	+++	
267	++++	
270	++	
271	++	
272	++	
276	++	
277	++	
278	++	
279	++++	
280	+++	
281	++	
282	++	
283	++	
284	++++	
289	++++	
290	+++	
291	++++	
292	++++	
293	++++	
294	++++	
295	+++	
296	++++	
297	+++	
298	++++	
300	++++	
301	++++	
302	++++	
303	++++	
304	++++	
305	++++	
306	++++	
307	+++	
308	++++	
309	+++	
310	++++	
312	++++	
313	++++	
314	++++	
315	++++	
316	++++	
317	++++	
318	++++	
319	++++	
320	++++	
321	++++	
322	++++	
323	++++	
324	++++	
326	++++	
327	++++	
328	++++	
329	++++	
330	++++	
331	++++	
332	++++	
333	++	
334	+++	
335	++++	
336	++++	
337	++++	
338	++++	
339	++++	
340	++++	

149

TABLE 5-continued

SP#	SJSA-1 EC50 (72 h)	
341	++++	
342	++++	5
343	++++	
344	++++	
345	++++	
346	++++	
347	++++	
348	++++	10
349	++++	
350	++++	
351	++++	
352	++++	
353	++++	
355	++++	15
357	++++	
358	++++	
359	++++	
360	++++	
361	+++	
362	++++	20
363	++++	
364	++++	
365	+++	
366	++++	
367	++++	
368	+	
369	++++	25
370	++++	
371	++++	
372	+++	
373	+++	
374	++++	
375	++++	30
376	++++	
377	++++	
378	++++	
379	++++	
380	++++	
381	++++	35
382	++++	
386	+++	
388	++	
390	++++	
392	+++	
394	+++	40
396	+++	
398	+++	
402	+++	
404	+++	
408	++++	
410	+++	
411	+++	45
412	+	
421	+++	
423	++++	
425	++++	
427	++++	
434	+++	50
435	++++	
436	++++	
437	++++	
438	++++	
439	++++	
440	++++	
441	++++	55
442	++++	
443	++++	
444	+++	
445	++++	
449	++++	
551	++++	60
552	++++	
554	+	
555	++++	
557	++++	
558	++++	
560	+	65
561	++++	

150

TABLE 5-continued

SP#	SJSA-1 EC50 (72 h)	
562	++++	
563	++++	
564	++++	
566	++++	
567	++++	
568	+++	
569	++++	
571	++++	
572	++++	
573	++++	
574	++++	
575	++++	
576	++++	
577	++++	
578	++++	
585	++++	
586	++++	
587	++++	
588	++++	
589	+++	
432	++++	
672	+	
673	++	
682	+	
686	+	
687	+	
662	++++	25
663	++++	
553	+++	
559	++++	
579	++++	
581	++++	
582	++	30
582	++++	
584	+++	
675	++++	
676	++++	
677	+	
679	++++	35
700	+++	
704	+++	
591	+	
706	++	
695	++	
595	++++	40
596	++++	
597	+++	
598	+++	
599	++++	
600	++++	
601	+++	
602	+++	45
603	+++	
604	+++	
606	++++	
607	++++	
608	++++	
610	++++	50
611	++++	
612	++++	
613	+++	
614	+++	
615	++++	
618	++++	
619	++++	55
707	++++	
620	++++	
621	++++	
622	++++	
623	++++	
624	++++	60
625	++++	
626	+++	
631	++++	
633	++++	
634	++++	
635	+++	65
636	+++	

151

TABLE 5-continued

SP#	SJSA-1 EC50 (72 h)
638	+
641	+++
665	++++
708	++++
709	+++
710	+
711	++++
712	++++
713	++++
714	+++
715	+++
716	++++
765	+
753	+
754	+
755	+
756	+
757	++++
758	+++

TABLE 6

SP#	HCT-116 EC50 (72 h)	RKO EC50 (72 h)	RKO-E6 EC50 (72 h)	SW480 EC50 (6 days)	IC50 Ratio
4	++++	++++	+++	++++	
5	++++	++++	+++	++++	
7	++++	++++	+++	++++	
10	++++	+++	+++	+++	
11	++++	++++	++	+++	
50	++++	++++	++	+++	
65	+++	+++	+++	+++	
69	++++	++++	+	++++	
70	++++	++++	++	+++	
71	++++	++++	+++	+++	
81	+++	+++	+++	+++	
99	++++	++++	+++	++++	
109	++++	++++	++	+++	
114		+++	+	+++	
115		+++	+	+++	1-29
118	+++	++++	+	++++	
120	++++	++++	+	++++	
121	++++	++++	+	++++	
122		+++	+	+++	1-29
125	+++	+++	+	+	
126	+	+	+	+	
148		++	+	+	
150		++	+	+	
153	+++		+		
154	+++	+++	+	+	30-49
158	+	+	+	+	
160	+++	+	+	+	1-29
161	+++	+	+	+	
175	+	+	+	+	
196	++++	++++	+++	++++	
219	++++	+++	+	+	1-29
233	++++				
237	++++		+	+	
238	++++		+	+	
243	++++		+	+	
244	++++		+	+	≥50
245	++++		+	+	
247	++++		+	+	
249	++++	++++	+	+	≥50
255	++++		+		
291			+		
293	+++		+		
303	+++		+		1-29
305			+		
306	++++		+		
310	++++		+		
312	++++				
313	++++		++		
314			+		

152

TABLE 6-continued

SP#	HCT-116 EC50 (72 h)	RKO EC50 (72 h)	RKO-E6 EC50 (72 h)	SW480 EC50 (6 days)	IC50 Ratio
315	++++	++++	++	++++	≥50
316	++++	++++	+	+++	≥50
317	+++		+	++	
321	++++		+		
324	+++		+		
325	+++				
326	+++		+		
327	+++				
328	+++		++		
329	++++		+		
330			+		
331	++++	++++	+	+	≥50
338	++++	++++	++	+++	
341	+++	++	+	+	
343	+++		+	+	
346	++++		+	+	
347	+++		+	+	
349	++++	+++	+	+	30-49
350	++++		+	+	
351	++++	+++	+	+	30-49
353		++	+	+	
355	++++	++	+	+	1-29
357	++++	++++	+	+	
358	++++	++	+	+	
359	++++	++	+	+	
367	++++		+	+	30-49
386	++++	++++	++++	++++	
388	++	++	+	+++	1-29
390	++++	++++	+++	++++	
435	+++	++	+		
436	++++	++++	++		
437	++++	++++	++	++++	30-49
440	++	++	+		
442	++++	++++	++		
444	++++	++++	+++		
445	++++	+++	+	+	≥50
555					≥50
557					≥50
558					30-49
562					30-49
564					30-49
566					30-49
567					≥50
572					≥50
573					30-49
578					30-49
662					≥50
379					1-29
375					1-29
559					≥50
561					1-29
563					1-29
568					1-29
569					1-29
571					1-29
574					1-29
575					1-29
576					1-29
577					30-49
433					1-29
551					30-49
553					1-29
710				+	
711				+	
712				++	
713				++	
714				+++	
715				+++	
716				+	

Example 11: P21 ELISA Assay

The assay was performed according to the following general protocol:

Cell Plating:

Trypsinize, count and seed SJSA1 cells at the density of 7500 cells/100 μ L/well in 96-well plates a day prior to assay.

On the day of study, replace media with fresh RPMI-11% FBS (assay media). Add 90 μ L of the assay media per well. Control wells with no cells, receive 100 μ L media.

Peptide Dilution:

Prepare 10 mM stocks of the peptides in DMSO. Serially dilute the stock using 1:3 dilution scheme to get 10, 3.3, 1.1, 0.33, 0.11, 0.03, 0.01 mM solutions using DMSO as diluents. Dilute the serially DMSO-diluted peptides 33.3 times using sterile water. This gives range of 10 $\times$ working stocks. Also prepare DMSO/sterile water (3% DMSO) mix for control wells.

Thus the working stocks concentration range μ M will be 300, 100, 30, 10, 3, 1, 0.3 and 0 μ M. Mix well at each dilution step using multichannel.

Row H has controls. H1-H3 will receive 10 μ L of assay media. H4-H9 will receive 10 μ L of 3% DMSO-water vehicle. H10-H12 will have media alone control with no cells.

Positive control: MDM2 small molecule inhibitor, Nutlin-3a (10 mM) is used as positive control. Nutlin was diluted using the same dilution scheme as peptides.

Addition of Working Stocks to Cells:

Add 10 μ L of 10 $\times$ desired concentration to appropriate well to achieve the final concentrations in total 100 μ L volume in well. (10 μ L of 300 μ M peptide+90 μ L of cells in media=30 μ M final concentration in 100 μ L volume in wells). Thus final concentration range used will be 30, 10, 3, 1, 0.3 & 0 μ M.

Controls will include wells that get no peptides but contain the same concentration of DMSO as the wells containing the peptides, and wells containing NO CELLS.

20 h-post incubation, aspirate the media; wash cells with 1 $\times$ PBS (without Ca⁺⁺/Mg⁺⁺) and lyse in 60 μ L of 1 $\times$ Cell lysis buffer (Cell Signaling technologies 10 $\times$ buffer diluted to 1 $\times$ and supplemented with protease inhibitors and Phosphatase inhibitors) on ice for 30 min.

Centrifuge plates in at 5000 rpm speed in at 4° C. for 8 min; collect clear supernatants and freeze at -80° C. till further use.

Protein Estimation:

Total protein content of the lysates is measured using BCA protein detection kit and BSA standards from Thermofisher. Typically about 6-7 μ g protein is expected per well.

Use 50 μ L of the lysate per well to set up p21 ELISA.

Human Total p21 ELISA:

The ELISA assay protocol is followed as per the manufacturer's instructions. 50 μ L lysate is used for each well, and each well is set up in triplicate.

Reagents:

Cell-Based Assay (-)-Nutlin-3 (10 mM): Cayman Chemicals, catalog #600034

OptiMEM, Invitrogen catalog #51985

Cell Signaling Lysis Buffer (10 $\times$), Cell signaling technology, Catalog #9803

Protease inhibitor Cocktail tablets (mini), Roche Chemicals, catalog #04693124001

Phosphatase inhibitor Cocktail tablet, Roche Chemicals, catalog #04906837001

Human total p21 ELISA kit, R&D Systems, DYC1047-5 STOP Solution (1M HCL), Cell Signaling Technologies, Catalog #7002

Instruments: Micro centrifuge—Eppendorf 5415D and Multiplate Reader for Absorbance readout (Synergy 2).

Example 12: Caspase 3 Detection Assay

The assay was performed according to the following general protocol:

Cell Plating:

Trypsinize, count and seed SJSA1 cells at the density of 7500 cells/100 μ L/well in 96-well plates a day prior to assay. On the day of study, replace media with fresh RPMI-11% FBS (assay media). Add 180 μ L of the assay media per well. Control wells with no cells, receive 200 μ L media.

Peptide Dilution:

Prepare 10 mM stocks of the peptides in DMSO. Serially dilute the stock using 1:3 dilution scheme to get 10, 3.3, 1.1, 0.33, 0.11, 0.03, 0.01 mM solutions using DMSO as diluents. Dilute the serially DMSO-diluted peptides 33.3 times using sterile water. This gives range of 10 $\times$ working stocks. Also prepare DMSO/sterile water (3% DMSO) mix for control wells.

Thus the working stocks concentration range μ M will be 300, 100, 30, 10, 3, 1, 0.3 and 0 μ M. Mix well at each dilution step using multichannel. Add 20 μ L of 10 $\times$ working stocks to appropriate wells.

Row H has controls. H1-H3 will receive 20 μ L of assay media. H4-H9 will receive 20 μ L of 3% DMSO-water vehicle. H10-H12 will have media alone control with no cells.

Positive control: MDM2 small molecule inhibitor, Nutlin-3a (10 mM) is used as positive control. Nutlin was diluted using the same dilution scheme as peptides.

Addition of working stocks to cells:

Add 10 μ L of 10 $\times$ desired concentration to appropriate well to achieve the final concentrations in total 100 μ L volume in well. (10 μ L of 300 μ M peptide+90 μ L of cells in media=30 μ M final concentration in 100 μ L volume in wells). Thus final concentration range used will be 30, 10, 3, 1, 0.3 & 0 μ M.

Controls will include wells that get no peptides but contain the same concentration of DMSO as the wells containing the peptides, and wells containing NO CELLS.

48 h-post incubation, aspirate 80 μ L media from each well; add 100 μ L Caspase3/7Glo assay reagent (Promega Caspase 3/7 glo assay system, G8092) per well, incubate with gentle shaking for 1 h at room temperature. read on Synergy Biotek multiplate reader for luminescence.

Data is analyzed as Caspase 3 activation over DMSO-treated cells.

Results from Examples 11 and 12 are shown in Table 7:

TABLE 7

SP#	caspase 0.3 μ M	caspase 1 μ M	caspase 3 μ M	caspase 10 μ M	caspase 30 μ M	p21 0.3 μ M	p21 1 μ M	p21 3 μ M	p21 10 μ M	p21 30 μ M
4			9	37	35			317	3049	3257
7	0.93	1.4	5.08	21.7	23.96		18	368	1687	2306
8			1	19	25			34	972	2857

TABLE 7-continued

SP#	caspase 0.3 μ M	caspase 1 μ M	caspase 3 μ M	caspase 10 μ M	caspase 30 μ M	p21 0.3 μ M	p21 1 μ M	p21 3 μ M	p21 10 μ M	p21 30 μ M
10	1		1	17	32		10	89	970	2250
11	1		5	23	33.5		140	350	2075.5	3154
26	1		1	3	14					
50			8	29	29		44	646	1923	1818
65	1		6	28	34	-69	-24	122	843	1472
69	4.34	9.51	16.39	26.59	26.11	272	458.72	1281.39	2138.88	1447.22
70			1	9	26		-19	68	828	1871
71	0.95	1.02	3.68	14.72	23.52		95	101	1204	2075
72	1		1	4	10	-19	57	282	772	1045
77	1		2	19	23					
80	1		2	13	20					
81	1		1	6	21		0	0	417	1649
99	1		7	31	33	-19	117	370	996	1398
109			4	16	25		161	445	1221	1680
114	1		6	28	34	-21	11	116	742	910
115	1		10	26	32	-10	36	315	832	1020
118	1		2	18	27	-76	-62	-11	581	1270
120	2		11	20	30	-4	30	164	756	1349
121	1		5	19	30	9	33	81	626	1251
122	1		2	15	30	-39	-18	59	554	1289
123	1		1	6	14					
125	1		3	9	29	50	104	196	353	1222
126	1		1	6	30	-47	-10	90	397	1443
127	1		1	4	13					
130	1		2	6	17					
139	1		2	9	18					
142	1		2	15	20					
144	1		4	10	16					
148	1		11	23	31	-23	55	295	666	820
149	1		2	4	10	35	331	601	1164	1540
150	2		11	19	35	-37	24	294	895	906
153	2		10	15	20					
154	2.68	4	13.93	19.86	30.14	414.04	837.45	1622.42	2149.51	2156.98
158	1		1.67	5	16.33	-1.5	95	209.5	654	1665.5
160	2		10	16	31	-43	46	373	814	1334
161	2		8	14	22	13	128	331	619	1078
170	1		1	16	20					
175	1		5	12	21	-65	1	149	543	1107
177	1		1	8	20					
183	1		1	4	8	-132	-119	-14	1002	818
196	1		4	33	26	-49	-1	214	1715	687
197	1		1	10	20					
203	1		3	12	10	77	329	534	1805	380
204	1		4	10	10	3	337	928	1435	269
218	1		2	8	18					
219	1		5	17	34	28	53	289	884	1435
221	1		3	6	12	127	339	923	1694	1701
223	1		1	5	18					
230	1		2	3	11	245.5	392	882	1549	2086
233	6	8	17	22	23	2000	2489	3528	3689	2481
237	1		5	9	15	0	0	2	284	421
238	1		2	4	21	0	149	128	825	2066
242	1		4	5	18	0	0	35	577	595
243	1		2	5	23	0	0	0	456	615
244	1		2	7	17	0	178	190	708	1112
245	1		3	9	16	0	0	0	368	536
247	1		3	11	24	0	0	49	492	699
248						0	50	22	174	1919
249	2		5	11	23	0	0	100	907	1076
251						0	0	0	0	0
252						0	0	0	0	0
253						0	0	0	0	0
254	1	3	7	14	22	118	896	1774	3042	3035
286	1	4	11	20	22	481	1351	2882	3383	2479
287	1	1	3	11	23	97	398	986	2828	3410
315	11	14.5	25.5	32	34	2110	2209	2626	2965	2635
316	6.5	10.5	21	32	32.5	1319	1718	2848	2918	2540
317	3	4	9	26	35	551	624	776	1367	1076
331	4.5	8	11	14.5	30.5	1510	1649	2027	2319	2509
338	1	5	23	20	29	660.37	1625.38	3365.87	2897.62	2727
341	3	8	11	14	21	1325.62	1873	2039.75	2360.75	2574
343	1	1	2	5	29	262	281	450	570	1199
346						235.86	339.82	620.36	829.32	1695.78
347	2	3	5	8	29	374	622	659	905	1567
349	1	8	11	16	24	1039.5	1598.88	1983.75	2191.25	2576.38
351	3	9	13	15	24	1350.67	1710.67	2030.92	2190.67	2668.54
353	1	2	5	7	30	390	490	709	931	1483

TABLE 7-continued

SP#	caspase 0.3 μ M	caspase 1 μ M	caspase 3 μ M	caspase 10 μ M	caspase 30 μ M	p21 0.3 μ M	p21 1 μ M	p21 3 μ M	p21 10 μ M	p21 30 μ M
355	1	4	11	13	30	191	688	1122	1223	1519
357	2	7	11	15	23	539	777	1080	1362	1177
358	1	2	3	6	24	252	321	434	609	1192
359	3	9	11	13	23	1163.29	1508.79	1780.29	2067.67	2479.29
416						33.74	39.82	56.57	86.78	1275.28
417						0	0	101.13	639.04	2016.58
419						58.28	97.36	221.65	1520.69	2187.94
432						54.86	68.86	105.11	440.28	1594.4

Example 13. Cell Lysis by Peptidomimetic
Macrocycles

SJSA-1 cells were plated out one day in advance in clear flat-bottom plates (Costar, catalog number 353072) at 7500 cells/well with 100 μ L/well of growth media, leaving row H columns 10-12 empty for media alone. On the day of the assay, media was exchanged with RPMI 1% FBS media, 90 μ L of media per well.

10 mM stock solutions of the peptidomimetic macrocycles were prepared in 100% DMSO. Peptidomimetic macrocycles were then diluted serially in 100% DMSO, and then further diluted 20-fold in sterile water to prepare working stock solutions in 5% DMSO/water of each peptidomimetic macrocycle at concentrations ranging from 500 μ M to 62.5 μ M.

10 μ L of each compound was added to the 90 μ L of SJSA-1 cells to yield final concentrations of 50 μ M to 6.25 μ M in 0.5% DMSO-containing media. The negative control (non-lytic) sample was 0.5% DMSO alone and positive control (lytic) samples include 10 μ M Melittin and 1% Triton X-100.

Cell plates were incubated for 1 hour at 37 C. After the 1 hour incubation, the morphology of the cells is examined by microscope and then the plates were centrifuged at 1200 rpm for 5 minutes at room temperature. 40 μ L of supernatant for each peptidomimetic macrocycle and control sample is transferred to clear assay plates. LDH release is measured using the LDH cytotoxicity assay kit from Caymen, catalog #1000882.

Results are shown in Table 8:

TABLE 8

SP#	6.25 μ M % Lysed cells (1 h LDH)	12.5 μ M % Lysed cells (1 h LDH)	25 μ M % Lysed cells (1 h LDH)	50 μ M % Lysed cells (1 h LDH)
3	1	0	1	3
4	-2	1	1	2
6	1	1	1	1
7	0	0	0	0
8	-1	0	1	1
9	-3	0	0	2
11	-2	1	2	3
15	1	2	2	5
18	0	1	2	4
19	2	2	3	21
22	0	-1	0	0
26	2	5	-1	0
32	0	0	2	0
39	0	-1	0	3
43	0	0	-1	-1
55	1	5	9	13
65	0	0	0	2
69	1	0.5	-0.5	5
71	0	0	0	0
72	2	1	0	3

TABLE 8-continued

SP#	6.25 μ M % Lysed cells (1 h LDH)	12.5 μ M % Lysed cells (1 h LDH)	25 μ M % Lysed cells (1 h LDH)	50 μ M % Lysed cells (1 h LDH)
75	-1	3	1	1
77	-2	-2	1	-1
80	0	1	1	5
81	1	1	0	0
82	0	0	0	1
99	1.5	3	2	3.5
108	0	0	0	1
114	3	-1	4	9
115	0	1	-1	6
118	4	2	2	4
120	0	-1	0	6
121	1	0	1	7
122	1	3	0	6
123	-2	2	5	3
125	0	1	0	2
126	1	2	1	1
130	1	3	0	-1
139	-2	-3	-1	-1
142	1	0	1	3
144	1	2	-1	2
147	8	9	16	55
148	0	1	-1	0
149	6	7	7	21
150	-1	-2	0	2
153	4	3	2	3
154	-1	-1.5	-1	-1
158	0	-6	-2	1
160	-1	0	-1	1
161	1	1	-1	0
169	2	3	3	7
170	2	2	1	-1
174	5	3	2	5
175	3	2	1	0
177	-1	-1	0	1
182	0	2	3	6
183	2	1	0	3
190	-1	-1	0	1
196	0	-2	0	3
197	1	-4	-1	-2
203	0	-1	2	2
204	4	3	2	0
211	5	4	3	1
217	2	1	1	2
218	0	-3	-4	1
219	0	0	-1	2
221	3	3	3	11
223	-2	-2	-4	-1
230	0.5	-0.5	0	3
232	6	6	5	5
233	2.5	4.5	3.5	6
237	0	3	7	55
243	4	23	39	64
244	0	1	0	4
245	1	14	11	56
247	0	0	0	4
249	0	0	0	0
254	11	34	60	75
279	6	4	5	6
280	5	4	6	18

TABLE 8-continued

SP#	6.25 μ M % Lysed cells (1 h LDH)	12.5 μ M % Lysed cells (1 h LDH)	25 μ M % Lysed cells (1 h LDH)	50 μ M % Lysed cells (1 h LDH)
284	5	4	5	6
286	0	0	0	0
287	0	6	11	56
316	0	1	0	1
317	0	1	0	0
331	0	0	0	0
335	0	0	0	1
336	0	0	0	0
338	0	0	0	1
340	0	2	0	0
341	0	0	0	0
343	0	1	0	0
347	0	0	0	0
349	0	0	0	0
351	0	0	0	0
353	0	0	0	0
355	0	0	0	0
357	0	0	0	0
359	0	0	0	0
413	5	3	3	3
414	3	3	2	2
415	4	4	2	2

Example 14: p53 GRIP Assay

Thermo Scientific* BioImage p53-MDM2 Redistribution Assay monitors the protein interaction with MDM2 and cellular translocation of GFP-tagged p53 in response to drug compounds or other stimuli. Recombinant CHO-hIR cells stably express human p53(1-312) fused to the C-terminus of enhanced green fluorescent protein (EGFP) and PDE4A4-MDM2(1-124), a fusion protein between PDE4A4 and MDM2(1-124). They provide a ready-to-use assay system for measuring the effects of experimental conditions on the interaction of p53 and MDM2. Imaging and analysis is performed with a HCS platform.

CHO-hIR cells are regularly maintained in Ham's F12 media supplemented with 1% Penicillin-Streptomycin, 0.5 mg/ml Geneticin, 1 mg/ml Zeocin and 10% FBS. Cells seeded into 96-well plates at the density of 7000 cells/100 μ l per well 18-24 hours prior to running the assay using culture media. The next day, media is refreshed and PD177 is added to cells to the final concentration of 3 μ M to activate foci formation. Control wells are kept without PD-177 solution. 24 h post stimulation with PD177, cells are washed once with Opti-MEM Media and 50 μ L of the Opti-MEM Media supplemented with PD-177 (6 μ M) is added to cells. Peptides are diluted from 10 mM DMSO stocks to 500 μ M working stocks in sterile water, further dilutions made in 0.5% DMSO to keep the concentration of DMSO constant across the samples. Final highest DMSO concentration is 0.5% and is used as the negative control. Cayman Chemicals Cell-Based Assay (–)–Nutlin-3 (10 mM) is used as positive control. Nutlin was diluted using the same dilution scheme as peptides. 50 μ l of 2 $\times$ desired concentrations is added to the appropriate well to achieve the final desired concentrations. Cells are then incubated with peptides for 6 h at 37° C. in humidified 5% CO₂ atmosphere. Post-incubation period, cells are fixed by gently aspirating out the media and adding 150 μ l of fixing solution per well for 20 minutes at room temperature. Fixed cells are washed 4 times with 200 μ l PBS per well each time. At the end of last wash, 100 μ l of 1 μ M Hoechst staining solution is added. Sealed plates incubated for at least 30 min in dark, washed with PBS to remove excess stain and PBS is added to each well. Plates

can be stored at 4° C. in dark up to 3 days. The translocation of p53/MDM2 is imaged using Molecular translocation module on Cellomics Arrayscan instrument using 10 $\times$ objective, XF-100 filter sets for Hoechst and GFP. The output parameters was Mean–CircRINGAveintenRatio (the ratio of average fluorescence intensities of nucleus and cytoplasm, (well average)). The minimally acceptable number of cells per well used for image analysis was set to 500 cells.

Example 15: MCF-7 Breast Cancer Study Using SP315, SP249 and SP154

A xenograft study was performed to test the efficacy of SP315, SP249 and SP154 in inhibiting tumor growth in athymic mice in the MCF-7 breast cancer xenograft model. A negative control stapled peptide, SP252, a point mutation of SP154 (F to A at position 19) was also tested in one group; this peptide had shown no activity in the SJSA-1 in vitro viability assay. Slow release 90 day 0.72 mg 173-estradiol pellets (Innovative Research, Sarasota, Fla.) were implanted subcutaneously (sc) on the nape of the neck one day prior to tumor cell implantation (Day –1). On Day 0, MCF-7 tumor cells were implanted sc in the flank of female nude (CrI: NU-Foxnlnu) mice. On Day 18, the resultant sc tumors were measured using calipers to determine their length and width and the mice were weighed. The tumor sizes were calculated using the formula (length $\times$ width²)/2 and expressed as cubic millimeters (mm³). Mice with tumors smaller than 85.3 mm³ or larger than 417.4 mm³ were excluded from the subsequent group formation. Thirteen groups of mice, 10 mice per group, were formed by randomization such that the group mean tumor sizes were essentially equivalent (mean of groups $\pm$ standard deviation of groups=180.7 $\pm$ 17.5 mm³).

SP315, SP249, SP154 and SP252 dosing solutions were prepared from peptides formulated in a vehicle containing MPEG(2K)-DSPE at 50 mg/mL concentration in a 10 mM Histidine buffered saline at pH 7. This formulation was prepared once for the duration of the study. This vehicle was used as the vehicle control in the subsequent study.

Each group was assigned to a different treatment regimen. Group 1, as the vehicle negative control group, received the vehicle administered at 8 mL/kg body weight intravenously (iv) three times per week from Days 18-39. Groups 2 and 3 received SP154 as an iv injection at 30 mg/kg three times per week or 40 mg/kg twice a week, respectively. Group 4 received 6.7 mg/kg SP249 as an iv injection three times per week. Groups 5, 6, 7 and 8 received SP315 as an iv injection of 26.7 mg/kg three times per week, 20 mg/kg twice per week, 30 mg/kg twice per week, or 40 mg/kg twice per week, respectively. Group 9 received 30 mg/kg SP252 as an iv injection three times per week.

During the dosing period the mice were weighed and tumors measured 1-2 times per week. Results in terms of tumor volume are shown in FIGS. 3-6 and tumor growth inhibition compared with the vehicle group, body weight change and number of mice with $\geq$ 20% body weight loss or death are shown in Table 9. Tumor growth inhibition (TGI) was calculated as % TGI=100–[(TuVol^{Treated-day x}–TuVol^{Treated-day18})/(TuVol^{Vehicle negative control-day x}–TuVol^{Vehicle negative control-day18})*100, where x=day that effect of treatment is being assessed. Group 1, the vehicle negative control group, showed good tumor growth rate for this tumor model.

For SP154, in the group dosed with 40 mg/kg twice a week 2 mice died during treatment, indicating that this

161

dosing regimen was not tolerable. The dosing regimen of 30 mg/kg of SP154 three times per week was well-tolerated and yielded a TGI of 84%.

For SP249, the group dosed with 6.7 mg/kg three times per week 4 mice died during treatment, indicating that this dosing regimen was not tolerable.

All dosing regimens used for SP315 showed good tolerability, with no body weight loss or deaths noted. Dosing with 40 mg/kg of SP315 twice per week produced the highest TGI (92%). The dosing regimens of SP315 of 26.7 mg/kg three times per week, 20 mg/kg twice per week, 30 mg/kg twice per week produced TGI of 86, 82, and 85%, respectively.

For SP252, the point mutation of SP154 which shows no appreciable activity in in vitro assays, dosing with 30 mg/kg three times per week was well-tolerated with no body weight loss or deaths noted. While TGI of 88% was noted by Day 32, that TGI was reduced to 41% by Day 39.

Results from this Example are shown in FIGS. 3-6 and are summarized in Table 9.

TABLE 9

Group Number	Treatment Group	% BW Change	No. with $\geq 10\%$ BW Loss	No. with $\geq 20\%$ BW Loss or death	% TGI
1	Vehicle	+8.6	0/10	0/10	—
2	SP154	+5.7	0/10	0/10	*84
3	SP154 30 mg/kg 3x/wk iv	N/A	0/10	2/10 (2 deaths)	Regimen not tolerated
4	SP249 6.7 mg/kg 3x/wk iv	N/A	6/10	4/10	Regimen not tolerated
5	SP315 26.7 mg/kg 3x/wk iv	+3.7	0/10	0/10	*86
6	SP315 20 mg/kg 2x/wk iv	+3.9	0/10	0/10	*82
7	SP315 30 mg/kg 2x/wk iv	+8.0	0/10	0/10	*85
8	SP315 40 mg/kg 2x/wk iv	+2.1	0/10	0/10	*92
9	SP252 30 mg/kg 3x/wk iv	+3.3	0/10	0/10	*41

*p $\leq$ 0.05 Vs Vehicle Control

162

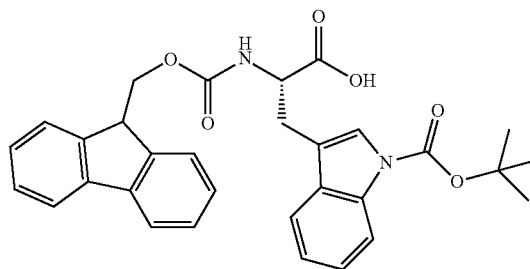
Example 21: Solubility Determination for Peptidomimetic Macrocycles

Peptidomimetic macrocycles are first dissolved in neat N, N-dimethylacetamide (DMA, Sigma-Aldrich, 38840-1L-F) to make 20 $\times$ stock solutions over a concentration range of 20-140 mg/mL. The DMA stock solutions are diluted 20-fold in an aqueous vehicle containing 2% Solutol-HS-15, 25 mM Histidine, 45 mg/mL Mannitol to obtain final concentrations of 1-7 mg/ml of the peptidomimetic macrocycles in 5% DMA, 2% Solutol-HS-15, 25 mM Histidine, 45 mg/mL Mannitol. The final solutions are mixed gently by repeat pipetting or light vortexing, and then the final solutions are sonicated for 10 min at room temperature in an ultrasonic water bath. Careful visual observation is then performed under hood light using a 7 $\times$ visual amplifier to determine if precipitate exists on the bottom or as a suspension. Additional concentration ranges are tested as needed to determine the maximum solubility limit for each peptidomimetic macrocycle.

Results from this Example are shown in FIG. 7.

Example 22: Preparation of Peptidomimetic Macrocycles Using a Boc-Protected Amino Acid

Peptidomimetic macrocycle precursors were prepared as described in Example 2 comprising an R8 amino acid at position “i” and an S5 amino acid at position “i+7”. The amino acid at position “i+3” was a Boc-protected tryptophan which was incorporated during solid-phase synthesis. Specifically, the Boc-protected tryptophan amino acid shown below (and commercially available, for example, from Novabiochem) was used during solid phase synthesis:



Metathesis was performed using a ruthenium catalyst prior to the cleavage and deprotection steps. The composition obtained following cyclization was determined by HPLC analysis to contain primarily peptidomimetic macrocycles having a crosslinker comprising a trans olefin (“iso2”, comprising the double bond in an E configuration). Unexpectedly, a ratio of 90:10 was observed for the trans and cis products, respectively.

SEQUENCE LISTING

The patent contains a lengthy “Sequence Listing” section. A copy of the “Sequence Listing” is available in electronic form from the USPTO web site (<https://seqdata.uspto.gov/?pageRequest=docDetail&DocID=US10967042B2>). An electronic copy of the “Sequence Listing” will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

163

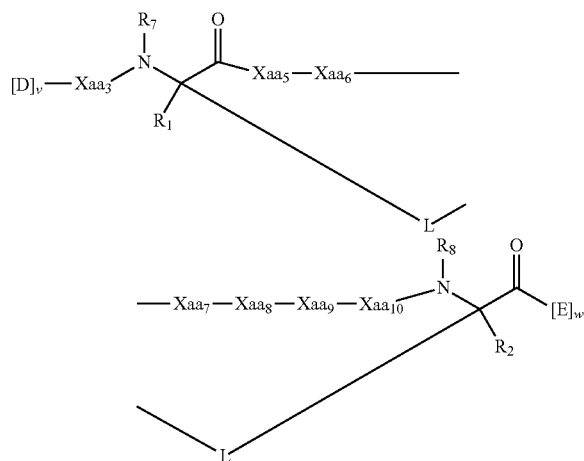
What is claimed is:

1. A pharmaceutical composition comprising, in a unit dosage form:

(a) a first therapeutic agent being an inhibitor of an interaction between p53 and MDM2 and/or an interaction between p53 and MDMX; and

(b) a second therapeutic agent being a chemotherapeutic agent,

wherein the first therapeutic agent is a peptidomimetic macrocycle of a Formula:



wherein:

each of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ is independently an amino acid, wherein at least three of Xaa₃, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉, and Xaa₁₀ are the same amino acids as the amino acid at the corresponding position of the sequence Phe₃-X₄-His₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀-X₁₁-Ser₁₂ (SEQ ID NO: 8) or Phe₃-X₄-Glu₅-Tyr₆-Trp₇-Ala₈-Gln₉-Leu₁₀/Cba₁₀-X₁₁-Ala₁₂ (SEQ ID NO: 9), wherein each X₄ and X₁₁ is independently an amino acid;

each D is independently an amino acid;

each E is independently an amino acid selected from the group consisting of Ala (alanine), D-Ala (D-alanine), Aib (α-aminoisobutyric acid), Sar (N-methyl glycine), and Ser (serine);

R₁ and R₂ are independently —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo-, or forms a macrocycle-forming linker L' connected to the alpha position of one of said D or E amino acids;

each L and L' is independently a macrocycle-forming linker;

each R₆ is independently —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heterocycloalkyl, a fluorescent moiety, a radioisotope or a therapeutic agent;

R₇ is —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, aryl, or heteroaryl, optionally substituted with R₅, or part of a cyclic structure with a D residue;

R₈ is —H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, aryl, or heteroaryl, optionally substituted with R₅, or part of a cyclic structure with an E residue;

164

each R₅ is independently halogen, alkyl, —OR₆, —N(R₆)₂, —SR₆, —SOR₆, SO₂R₆, —CO₂R₆, a fluorescent moiety, a radioisotope or a therapeutic agent;

v is an integer from 1-10; and

w is an integer from 3-10.

2. The pharmaceutical composition of claim 1, wherein the unit dosage is formulated for administering intravenously.

3. The pharmaceutical composition of claim 1, wherein the unit dosage is formulated for administering intraperitoneally.

4. The pharmaceutical composition of claim 1, wherein the unit dosage is formulated for administering subcutaneously.

5. The pharmaceutical composition of claim 1, wherein the peptidomimetic macrocycle comprises an amino acid sequence with at least about 60% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 10-457, or a pharmaceutically acceptable salt thereof.

6. The pharmaceutical composition of claim 1, wherein L and L' are independently a macrocycle-forming linker of the formula —L₁-L₂—, wherein:

L₁ and L₂ are independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, heteroarylene, or [—R₄—K—R₄—]_n, each being optionally substituted with R₅;

each K is independently O, SO, SO₂, CO, CO₂, or CONR₃;

each R₃ is independently hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, aryl, or heteroaryl, optionally substituted with R₅;

each R₄ is independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, or heterocycloalkylene; and

each n is independently an integer from 1-5.

7. The pharmaceutical composition of claim 1, wherein L is alkylene, alkenylene, or alkynylene.

8. The pharmaceutical composition of claim 1, wherein R₁ and R₂ are independently alkyl.

9. The pharmaceutical composition of claim 8, wherein R₁ and R₂ are methyl.

10. The pharmaceutical composition of claim 1, wherein R₁ and R₂ are —H.

11. The pharmaceutical composition of claim 1, wherein v is an integer from 2-5.

12. The pharmaceutical composition of claim 1, wherein w is an integer from 3-6.

13. The pharmaceutical composition of claim 1, wherein [D]_v is —Leu₁-Thr₂.

14. The pharmaceutical composition of claim 1, wherein the peptidomimetic macrocycle is present in the unit dosage form in an amount from about 0.1 mg/kg to about 50 mg/kg.

15. The pharmaceutical composition of claim 1, wherein the peptidomimetic macrocycle is present in the unit dosage form in an amount from about 6.7 mg/kg to about 40 mg/kg.

16. The pharmaceutical composition of claim 1, further comprising a pharmaceutically-acceptable excipient.

17. The pharmaceutical composition of claim 1, wherein the peptidomimetic macrocycle comprises at least one alpha-helix.

18. The pharmaceutical composition of claim 1, wherein the unit dosage form is a capsule, tablet, cachet, or lozenge.

* * * * *